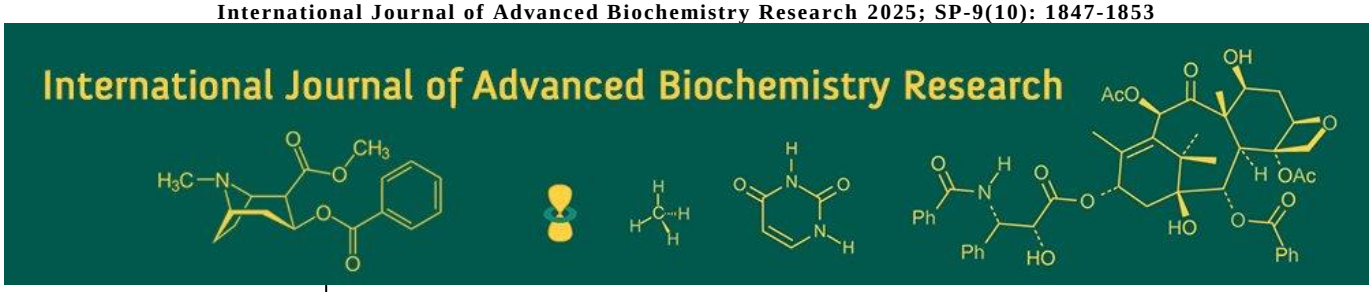

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Identification of resistant bitter gourd (*Momordica charantia* L.) genotypes against CMV, ToLCNDV, and PRSV mosaic viruses using graft inoculation and biochemical-serological approaches

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Abstract
Bitter gourd production faces severe constraints due to mosaic disease caused by a viral complex comprising Cucumber Mosaic Virus (CMV), Tomato Leaf Curl New Delhi Virus (ToLCNDV), and Papaya Ringspot Virus (PRSV). This study sought to identify and characterize naturally resistant genotypes for integration into breeding programs aimed at developing virus-resistant varieties. Thirty bitter gourd genotypes were evaluated after artificial inoculation via wedge grafting, an efficient technique for simultaneous transmission of multiple viruses. Disease response was quantified using the Vulnerability Index (VI) and a 0-5 symptom severity scale. Three landraces—Lodhi local, Udayagiri local, and Therthari local—demonstrated high resistance (VI = 0%) with no observable symptoms. In contrast, commercial cultivars such as Priyanka and Preethi showed high susceptibility (VI up to 86.67%). Biochemical profiling reve aled that resistant genotypes exhibited elevated baseline and induced activity of defense enzymes—peroxidase (PER), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL)—compared to susceptible lines. These findings were further validated using Double Antibody Sandwich ELISA (DAS-ELISA), which confirmed the absence of viral antigens in the highly resistant genotypes, as indicated by low absorbance values. The identified resistant genotypes represent a valuable genetic resource for developing resilient bitter gourd varieties, which is essential for sustainable disease management and improving crop yield.

Keywords: Bitter gourd, *Momordica charantia*, CMV, ToLCNDV, PRSV, DAS-ELISA

Introduction
Bitter gourd (*Momordica charantia* L.), also known as bitter melon, is a key cucurbit vegetable extensively cultivated in Kerala. The crop is thought to have originated in the tropical regions of the Old World, particularly eastern India and southern China (Garrison, 1977) [14]. As a monoecious, highly cross-pollinated diploid crop (2n=22), bitter gourd is renowned for its nutritional and therapeutic benefits. The fruits are abundant in alkaloid compounds like momordicine, saponine, and albuminoides, as well as high levels of vitamin C and folate. Beyond their culinary applications, these fruits possess various pharmacological properties, including antioxidant, anti-diabetic, antibacterial, and anti-cancer effects (Fuangchan *et al*., 2011) [13].

Despite its economic value, bitter gourd production faces significant challenges from biotic and abiotic stresses, particularly viral infections. Viruses induce complex defense responses in plants, often at a high metabolic cost, leading to reduced yields (Syller and Grupa, 2016) [32]. Among these, mosaic disease—predominantly active in summer—has emerged as a major constraint. Over 35 distinct viruses have been identified in cucurbits (Provident, 1996) [26], with Cucumber mosaic virus (CMV) (Nagarajan and Ramakrishnan, 1971; Akbar *et al*., 2015) [21, 2], Papaya ringspot virus (PRSV) (Rajinimala *et al*., 2005; Kumar *et al*., 2021), and Tomato leaf curl New Delhi virus (ToLCNDV) (Tiwari *et al*., 2010; Naik *et al*., 2022) [34, 23] being the most prevalent in Kerala. These viruses may occur individually or as mixed infections (Nameth *et al*., 1986) [24], causing symptoms such as yellow mosaic, leaf curling, stunted growth, and reduced leaf size (Nagendran *et al*., 2017; Gomathi *et al*., 2023) [22, 15].

Nevertheless, the commercial production of bitter gourd faces numerous challenges from both biotic and abiotic factors, with viral infections being a significant threat. Viruses are

obligate intracellular parasites, and no therapeutic treatments have been reported, complicating field management (Nicaise, 2014) [25]. High-yielding varieties are particularly vulnerable, and the disease is mainly transmitted by insect pests such as aphids and whiteflies. Once a plant is infected, the primary management strategy is to control the vectors to prevent further disease spread, often resulting in the excessive use of insecticides with associated health and environmental risks. Therefore, healthy planting materials and disease-resistant cultivars are essential for sustainable bitter gourd cultivation. An integrated disease management system, tailored to different agro-ecological conditions and combined with resistant crop varieties, provides a more effective and environmentally friendly solution to address viral epidemics (Martín-Hernández and Picó, 2021) [19].

In light of these challenges, this study focuses on evaluating bitter gourd germplasm to identify mosaic-resistant genotypes for use in future breeding programs aimed at enhancing crop resilience.

Materials and Methods

The experiment utilized 30 bitter gourd genotypes, comprising both *M. charantia* var. charantia and *M. charantia* var. *muricata*, which encompassed released varieties from KAU, national varieties, accessions from the National Bureau of Plant Genetic Resources (NBPGR), and local cultivars (Table 1). The study was conducted at Farming Systems Research Station, Sadananadapuram, Kottarakara, Kerala. Specifically, the 30 genotypes included two released varieties from KAU, 13 accessions from NBPGR, and the remaining genotypes were locally sourced.

Table 1: List of bitter gourd genotypes

No.	Varieties	Source
T_1	Priyanka	Kerala Agricultural University
T_2	Bangalore Local	Karnataka
T_3	Erumely Local	Kottayam,
T_4	Kattapana Local	Idukki
T_5	Udayagiri Local	Udayagiri
T_6	Therthali Local	Therthali
T_7	Lodhi Local	New delhi
T_8	Palakkad Local	Palakkad
T_9	Thiruvananthapuram Local	Thiruvananthapuram
T_{10}	Wadakkanchery Local	Palakkad
T_{11}	Omalloor Local	Pathanamthitta
T_{12}	Punjab Local	Punjab
T_{13}	Kollam Local	Kollam
T_{14}	Preethi	Kerala Agricultural University
T_{15}	Thrissur Local	Thrissur
T_{16}	Salem Local	Tamil nadu
T_{17}	IC 44413	NBPGR, New Delhi
T_{18}	IC 44418	NBPGR, New Delhi
T_{19}	IC 44419	NBPGR, New Delhi
T_{20}	IC 44423	NBPGR, New Delhi
T_{21}	IC 44424	NBPGR, New Delhi
T_{22}	IC 44426	NBPGR, New Delhi
T_{23}	IC 68275	NBPGR, New Delhi
T_{24}	IC 68335	NBPGR, New Delhi
T_{25}	IC 470558	NBPGR, New Delhi
T_{26}	IC 433630	NBPGR, New Delhi
T_{27}	IC 596980	NBPGR, New Delhi
T_{28}	IC 599429	NBPGR, New Delhi
T_{29}	IC 599434	NBPGR, New Delhi
T_{30}	IC 541436	NBPGR, New Delhi

1. Artificial screening of germplasm for mosaic complex tolerance.

Screening of 30 genotypes for mosaic tolerance by graft transmission of the viruses was done. Wedge grafting was performed to transfer viruses to healthy plants. This method was chosen for its higher success rate in grafting. The different genotypes were used as rootstock and the susceptible variety Preethi, which carries the three viruses, were used as scions. A wedge or 'V' shaped incision was made on the rootstock, about one centimeter above the cotyledonary leaves, to insert the infected scion. The scion was prepared by creating a tapered cut at its end to fit into the cut of the rootstock. A graft clip was used to secure the graft union. The grafted plants were covered with a clear plastic bag for protection. The young shoots that developed from the axils of the cotyledonary leaves of the rootstock were monitored for the appearance of symptoms. Symptom severity was evaluated using a 0-5 scale, adapted from Bos (1982) [10] and modified specifically for assessing viral disease in bitter gourd.

1. No symptom
2. Very light mottling
3. Mottling with dark green and yellow colour
4. Blisters and raised surface on leaves
5. Distortion of leaves, curling, hairiness, reduction in leaf size
6. Stunting with negligible or no flowering and fruiting or very small fruits

Based on the scoring, Vulnerability Index (VI) was found out with the formula,

$$\text{Vulnerability index} = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5) \times 100}{n_t\,(n_c - 1)}$$

n_0, $n_{1\ldots}$ n_5 - Number of plants in the category of 0, 1, 2,3,4,5
n_t - Total number of plants
n_c - Number of categories

Percent disease incidence (PDI) was analyzed using the formula,

$$\text{Percent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

2. Biochemical analysis

Biochemical analysis of grafted plants was carried out. Analysis included estimation of defense related enzymes such as peroxidase (PER), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL).

Peroxidase activity was determined according to the method described by Srivastava (1987). One gram leaf sample was homogenized in 5 ml of 0.1 M sodium phosphate buffer (pH 6.5) with a pinch of polyvinyl pyrrolidone (PVP) added to it. The sample was homogenized in a mortar and pestle at 40 °C, then centrifuged for 15 minutes at 5000 rpm and 40 °C after filtering through a muslin cloth. The resulting supernatant was used as the enzyme extract. To measure peroxidase (PO) activity, a reaction mixture consisting of 1 ml of 0.05 M pyrogallol and 50 µl of enzyme extract was prepared in a sample cuvette, while pyrogallol alone was used in a reference cuvette. The spectrophotometer was set to zero at 420 nm, and the reaction was initiated by adding 1 ml of 1% hydrogen peroxide (H_2O_2) to the sample cuvette.

The change in absorbance was then measured at 30-second intervals up to 180 seconds.

Polyphenol oxidase (PPO) activity was determined according to the method of Mayer *et al*. (1965) [20]. The enzyme extraction procedure was identical to that used for peroxidase. The reaction mixture consisted of 1 ml of 0.1M sodium phosphate buffer (pH 6.5) and 50 µl of enzyme extract in the sample cuvette, while the reference cuvette contained only sodium phosphate buffer. The spectrophotometer was set to zero at 495 nm. The reaction was initiated by adding 1 ml of 0.01M pyro-catechol to the sample cuvette, and the change in absorbance was measured at 30-second intervals up to 180 seconds. The PPO activity was expressed as the change in absorbance per minute per gram of fresh weight.

The PAL activity was investigated using the method described by Dickerson *et al*. (1984) [12]. To prepare the enzyme extract, 1g of leaf sample was homogenized in 5ml of sodium borate buffer (pH 8.8) containing a small amount of PVP using a chilled mortar and pestle. The homogenate was then centrifuged at 10,000 rpm for 10 minutes at 40 °C, and the resulting supernatant was used to measure PAL activity. The reaction mixture consisted of 3ml of sodium borate buffer, 0.2ml of enzyme extract, and 0.1ml of 12mM 1-phenylalanine prepared in the same buffer. A blank sample was also prepared by combining 0.2ml of enzyme extract with 3ml of sodium borate buffer. After incubating the reaction mixture and blank at 40 °C for 30 minutes, the reaction was stopped by adding 0.2ml of 3N hydrochloric acid. The absorbance was then measured at 290nm using a spectrophotometer, and PAL activity was expressed as micrograms of cinnamic acid produced per minute per gram of fresh weight.

3. Screening of resistant genotypes identified using DAS-ELISA

A Double Antibody Sandwich ELISA was conducted to determine the presence or absence of Cucumber mosaic virus (cucumovirus), Tomato Leaf Curl New Delhi Virus (begomovirus), and Papaya Ring Spot Virus (potyvirus) in inoculated bitter gourd plants. The ELISA procedure followed the protocol outlined by the DSMZ Plant Virus Department in Braunschweig, Germany (Clark and Adams, 1977) [11].

After recommended dilution, 200 µl of antibody was added to each well of a microtiter plate, which was then covered and incubated for 2 to 4 hours at 37 °C. The plate was then washed three times with PBS-Tween and blotted dry. Next, leaf samples (1g) were homogenized in 5ml of a specific extraction buffer, depending on the virus being tested (PBS-Tween with 2% PVP for CMV and PRSV, or 0.05 M Tris with 0.06 M sodium sulfite for ToLCNDV). The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4 °C. A 200 µl aliquot of the resulting supernatant was added in duplicate to the washed wells and incubated overnight at 4 °C. After another three washes, 200 µl of diluted enzyme conjugate was added to each well and incubated for 2-4 hours at 37 °C, followed by a final three washes. Finally, 200 µl of a freshly prepared substrate was added to each well and incubated at 37 °C for 30-60 minutes. The absorbance was measured at 405 nm using an ELISA reader. A sample was considered infected if a yellow color developed and its optical density (OD) value was more than twice that of the negative control.

Results and Discussion

1. Artificial screening of germplasm for mosaic complex tolerance

The study entailed the assessment of 30 bitter gourd genotypes following their artificial inoculation with mosaic-inducing viruses, namely Cucumber Mosaic Virus (CMV), begomovirus- Tomato Leaf Curl New Delhi Virus (ToLCNDV), and potyvirus- Papaya Ringspot Virus (PRSV). Transmission of begomovirus occurs via whiteflies, whereas CMV and PRSV are vectored by aphids. To facilitate the simultaneous inoculation of these three viruses into healthy plants, separate transmission methods involving whiteflies and aphids would typically be necessary. However, the technique of wedge grafting enables the concurrent transmission of all three viruses by establishing a phloem connection. Consequently, wedge grafting was selected as the preferred method for virus transmission in this research. According to Radhika (2018) [27], wedge grafting proves to be a more dependable approach than the individual use of whiteflies and aphids for inoculating begomovirus, CMV, and PRSV in bitter gourd.

Post artificial screening, symptom manifestation was quantified using a 0-5 scale, in accordance with the Vulnerability Index proposed by Bos (1982) [10]. The mosaic complex in bitter gourd is predominantly caused by a combination of viral infections in the field, with Cucumber Mosaic Virus (CMV), Papaya Ringspot Virus (PRSV), and Tomato Leaf Curl New Delhi Virus (ToLCNDV) commonly co-occurring (Tiwari *et al.*, 2010) [34]. Symptoms were most pronounced on the leaves of secondary branches at the plant's apex. Initially, the disease manifested as irregular, scattered yellow spots on a few leaves. As the disease progressed, vein clearing was observed in isolated sections of one or two leaves. In severe cases, affected plants showed a decrease in leaf size, with elongation or stunting in certain leaf areas. New leaves exhibited deformities and were smaller in size. Some leaves displayed reduced lamina growth, resulting in a characteristic shoestring-like appearance.

Certain genotypes manifested symptoms typical of Cucumber Mosaic Virus (CMV), including vein clearing, downward curling of leaf margins, and a thickened, leathery texture (Nagarajan and Ramakrishnan, 1971) [21]. Simultaneously, numerous genotypes showed symptoms such as vein clearing, thinner and smaller leaves, and yellowing, indicative of Potyvirus infections (Ashwini, 2015) [6]. Additionally, symptoms associated with Tomato Leaf Curl New Delhi Virus (ToLCNDV) were noted on some leaves, starting as small yellow spots along the leaf margins that quickly spread across the entire leaf, leading to distortion and size reduction. Collectively, these viral diseases produce characteristic symptoms such as mosaic patterns on leaves, reduced leaf size, leaf crinkling and deformation, yellow veins, enations on the leaf undersides, overall plant stunting, and yellow mosaic patches. In several documented instances, the presence of these viruses has resulted in total (100%) yield loss (Nagendran *et al.*, 2017; Kiran *et al.*, 2021) [22, 16] (Figure 1).

Among the evaluated bitter gourd germplasm (n=30), three landrace accessions - Lodhi local (var. *charantia*), Udayagiri local (var. *muricata*), and Therthali local (var. *muricata*) - demonstrated complete resistance (VI=0%) to the viral complex comprising CMV, ToLCNDV, and PRSV. These genotypes were consequently categorized as resistant

based on their immunity to infection. The Vulnerability Index, as defined by Bos (1982) [10], served as a quantitative measure of susceptibility to mosaic disease, with higher values indicating greater vulnerability and lower values signifying increased resistance (Table 2).

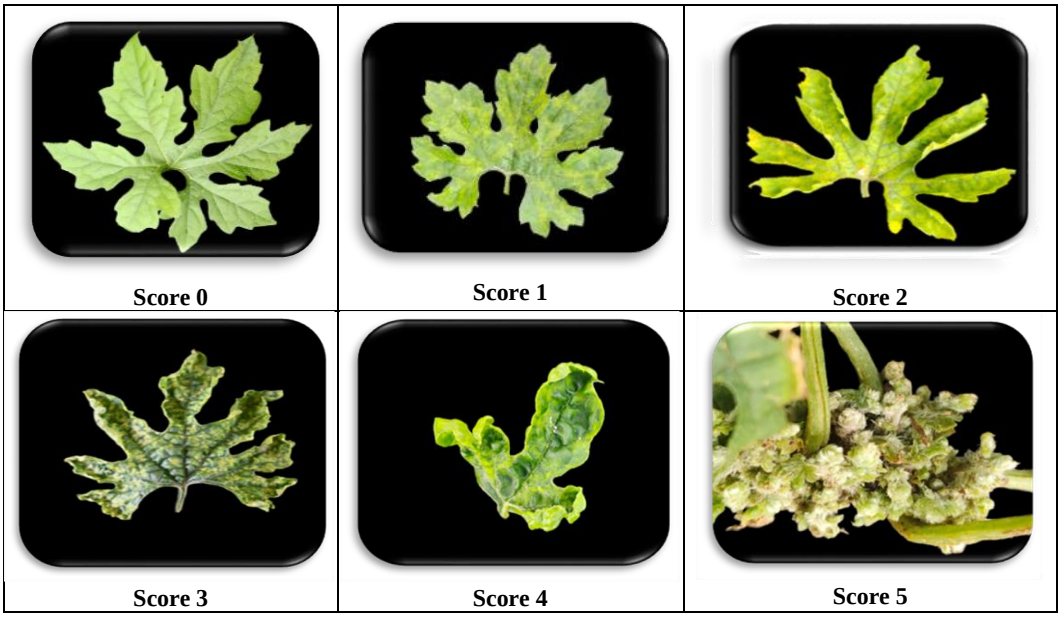

Fig 1: Scoring of mosaic symptoms

Table 2: Category of disease reaction based on Vulnerability Index (VI) value

Vulnerability Index (%)	Disease reaction
0 to 5	Highly Resistant (HR)
5.1 to 10	Resistant (R)
10.1 to 20	Moderately Resistant (MR)
20.1 to 40	Moderately Susceptible (MS)
40.1 to 70	Susceptible (S)
70.1 to 100	Highly susceptible (HS)

Priyanka displayed the highest VI (86.67%), comparable to Wadakkanchery local (83.33%), indicating a high degree of susceptibility to viral infection in these genotypes. Several genotypes, including Priyanka, Kottayam local, Wadakkanchery local, and Salem local, exhibited complete disease incidence (100% PDI). Conversely, Lodhi local, Udayagiri local, and Therthali local remained asymptomatic, demonstrating 0% PDI (Table 3).

Table 3: Vulnerability Index and Percentage Disease Incidence of the genotypes (*Value in parentheses are square root transformed)

Treatments	Vulnerability Index (VI)	Percentage Disease Incidence (PDI)	Disease Score	Disease Reaction
T_1	86.67 (9.335)	100	5	HS
T_2	63.33 (7.988)	90	4	S
T_3	66.67 (8.194)	100	4	S
T_4	30.00 (5.517)	50	3	MS
T_5	0.00 (0.707)	0	0	HR
T_6	0.00 (0.707)	0	0	HR
T_7	0.00 (0.707)	0	0	HR
T_8	46.67 (6.865)	80	4	S
T_9	43.33 (6.617)	90	4	S
T_{10}	83.33 (9.155)	100	5	HS
T_{11}	50.00 (7.104)	90	4	S
T_{12}	10.00 (3.212)	40	1	R
T_{13}	36.67 (6.092)	60	3	MS
T_{14}	73.33 (8.591)	90	5	HS
T_{15}	60.00 (7.776)	90	4	S
T_{16}	80.00 (8.971)	100	5	HS
T_{17}	76.66 (8.783)	90	5	HS
T_{18}	46.67 (6.865)	80	4	S
T_{19}	63.33 (7.988)	100	4	S
T_{20}	56.66 (7.559)	90	4	S
T_{21}	50.00 (7.104)	90	4	S
T_{22}	76.66 (8.783)	90	5	HS
T_{23}	70.00 (8.395)	80	4	S

T_{24}	16.67 (4.13)	60	2	MR
T_{25}	23.33 (4.874)	50	3	MS
T_{26}	66.67 (8.194)	100	4	S
T_{27}	33.33 (5.812)	80	3	MS
T_{28}	13.33 (3.701)	70	2	MR
T_{29}	43.33 (6.617)	90	4	S
T_{30}	73.33 (8.591)	100	5	HS

The assessment of bitter gourd genotypes for their response to viral infections was conducted using a disease rating scale, as documented by Arunachalam (2002) [5], Resmi (2009) [28], Ashwini (2015) [6], and Asna (2018) [17]. In a study by Asna *et al.* (2018) [17], 53 accessions of bitter gourd were evaluated for resistance to a viral disease, and it was found that no genotype displayed total immunity. In the present investigation, the KAU varieties Priyanka and Preethi were identified as highly susceptible, whereas in previous research by Ashwini (2015) [6] and Asna *et al.* (2018) [17], they were classified merely as susceptible. The outcomes of experiments by Resmi and Sreelathakumary, Radhika (2018) [27], and Ankitha (2024) [3] corroborate these current observations. Over successive generations, genotypes may lose their resistance to infection due to the degradation of resistance mechanisms or the emergence of new viral strains capable of overcoming existing resistance. The current results suggest that the identified virus-resistant genotypes represent valuable resources for future research aimed at elucidating the molecular basis of resistance and for genetic improvement programs.

2. Biochemical analysis

Resistant bitter gourd genotypes exhibited upregulated synthesis of key defense-related enzymes, including peroxidase (PER), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL), whereas susceptible varieties such as Bangalore local, Omalloor local, and IC 44423 showed diminished enzymatic activity. Comparative analysis revealed significantly higher baseline levels of these enzymes in resistant plants relative to susceptible counterparts, consistent with their role in antiviral defense mechanisms. Enhanced production of these enzymes during viral infection reflects a coordinated biochemical response to inhibit viral replication and systemic spread. This defense network is typically activated through plant-pathogen recognition pathways, wherein viral components or cellular stress signals trigger molecular cascades that bolster resistance (Table 4).

The results from the present investigation are consistent with the observations made by Thangamani *et al.* (2011) [33], where enzyme activity was found to be highest in resistant hybrids, followed by moderately resistant hybrids, moderately susceptible ones, and lowest in susceptible genotypes. A comparable trend was noted in research conducted by Kumar *et al.* (2023) [17] on potatoes, where infection by Tomato Leaf Curl New Delhi Virus (ToLCNDV) resulted in a marked elevation of peroxidase activity in resistant cultivars. Peroxidases are essential in counteracting reactive oxygen species (ROS) generated during the plant's defense response (Sharma *et al.*, 2021) [30]. ROS can inflict damage on plant cells, and peroxidases assist in alleviating this damage, thus lessening the cellular impact. Astaraki and Shams-bakhsh (2023) [9] also reported that peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase enzymes act as resistance factors due to their elevated activities in resistant cultivars relative to susceptible ones. These findings have been further substantiated by Ankitha (2024) [3], highlighting the pivotal role of antioxidant enzymes in mitigating viral pathogenesis and maintaining plant health amidst biotic stress.

Table 4: Estimation of defense enzymes in bitter gourd genotypes

Genotypes	PER (µg g^{-1} min^{-1})	PPO (µg g^{-1} min^{-1})	PAL (µg g^{-1} min^{-1})
T_1	7.267	2.14	55.77
T_2	5.433	2.00	50.89
T_3	6.523	2.52	53.56
T_4	6.893	2.94	56.14
T_5	9.043	3.94	61.33
T_6	9.287	4.01	61.75
T_7	10.113	4.27	62.08
T_8	6.04	1.77	56.61
T_9	6.567	1.82	53.15
T_{10}	5.63	1.94	52.36
T_{11}	6.58	1.66	53.52
T_{12}	8.417	3.13	58.44
T_{13}	5.897	2.64	53.11
T_{14}	6.747	1.81	54.35
T_{15}	6.513	2.00	54.57
T_{16}	5.75	1.99	52.64
T_{17}	6.343	1.83	50.45
T_{18}	6.343	2.23	52.63
T_{19}	6.123	2.34	51.50
T_{20}	6.17	2.40	50.38
T_{21}	5.997	2.32	52.10
T_{22}	6.357	1.79	50.66
T_{23}	7.86	3.18	57.75
T_{24}	6.49	2.64	53.35

T_{25}	5.457	2.45	51.77
T_{26}	7.25	2.26	52.79
T_{27}	5.067	2.05	52.82
T_{28}	7.753	2.77	54.90
T_{29}	5.437	1.98	50.80
T_{30}	6.34	1.82	50.64
SE(m)	0.059	0.074	0.181
CD(5%)	0.166	0.21	0.512

3. Screening of resistant genotypes identified using DAS-ELISA

The presence or absence of Cucumber Mosaic Virus, begomovirus- Tomato Leaf Curl New Delhi Virus, and potyvirus- Papaya Ringspot Virus in inoculated bitter gourd plants was determined using Double Antibody Sandwich ELISA (DAS-ELISA), following the protocol established by the DSMZ Plant Virus Department, Braunschweig, Germany.

Post-inoculation evaluation revealed no symptomatic expression of the three viruses in Udayagiri local (T_5), Therthali local (T_6) and Lodhi local (T_7). Udayagiri local had the lowest absorbance values for CMV at 0.009, PRSV at 0.013 and ToLCNDV at 0.013. In ELISA, the absorbance value is directly correlated with the concentration of the virus; a higher absorbance indicates a greater presence of the virus. The DAS-ELISA results showed that all genotypes identified as highly resistant had absorbance values less than double those of healthy, non-infected plants. These resistant genotypes remained free of symptoms throughout the experiment due to their inherent resistance mechanisms (Table 5).

The Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) has been widely established as a reliable method for viral pathogen detection in cucurbit species. For instance, Abdelkhalek *et al.* (2022) [1] confirmed its utility in diagnosing Cucumber Mosaic Virus (CMV) in squash, whereas Yazdani-Khameneh *et al.* (2016) [35] applied the technique to identify Tomato Leaf Curl New Delhi Virus (ToLCNDV) in cucurbitaceous crops. Consistent with these findings, Ankitha *et al.* (2023) [4] validated DAS-ELISA's effectiveness in bitter gourd viral screening. Gomathi Devi (2023) [15] further expanded its application to seed diagnostics, reporting embryo-specific optical density (OD) values of 0.24-1.50 in begomovirus-infected bitter gourd seeds against a control value of 0.54, emphasizing embryonic viral load as a pivotal factor in seed-mediated transmission. Similarly, Kumar *et al.* (2014) [18] observed elevated OD values (0.566 vs. 0.204 in controls) in PRSV-infected snake gourd tissues, corroborating absorbance trends across studies. These observations align with Kumar *et al.* (2023) [17], who advocated for the synergistic application of ELISA and PCR methodologies to enhance diagnostic precision in plant virology, underscoring their combined efficacy in pathogen detection and confirmation.

Table 5: ELISA absorbance values of the highly resistant genotypes

Genotype	CMV(A405) value	Increase in absorbance	ToLCNDV (A405)value	Increase in absorbance	PRSV (A405)value	Increase in absorbance
Lodhi local	0.009	1.125	0.016	1.33	0.013	0.7
Udayagiri local	0.013	1.625	0.013	1.08	0.013	0.7
Therthali local	0.011	1.375	0.017	1.42	0.015	1.5
Un-inoculated healthy plants	0.008	-	0.012	-	0.010	-

Conclusion

This study systematically screened 30 bitter gourd genotypes for resistance to a three-virus mosaic disease complex (CMV, ToLCNDV, and PRSV). Using wedge-grafting to synchronize infection, we identified three genotypes—Lodhi local, Udayagiri local, and Therthali local—that exhibited high resistance (Vulnerability Index = 0). Biochemical profiling showed these lines had higher constitutive levels and stronger induction of defense enzymes (peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase) than susceptible checks. DAS-ELISA corroborated the resistance, detecting little to no virus based on low absorbance. These genotypes represent strong candidates as donors for breeding virus-resistant bitter gourd varieties.

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