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Varietal Reaction of *Momordica charantia* Germplasm to Viruses Infecting Bitter Gourd

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ABSTRACT: Diseases caused by viruses are among the most limiting factors for bitter gourd (*Momordica charantia* L.) production in India, which can reach up to 100% crop loss. Severe outbreaks of viral diseases can occur when there is a suitable environment for both the virus and its vector, combined with a lack of awareness about the diseases. The present study was conducted to screen out available bitter gourd germplasm against mosaic viruses. Reactions of 33 bitter gourd accessions belonging to *Momordica charantia* var. *charantia* and *Momordica charantia* var. muricata were studied by artificial inoculation with three viruses namely, *Cucumber Mosaic Virus* (CMV), *Tomato Leaf Curl New Delhi Virus* (ToLCNDV) and *Papaya Ring Spot Virus* (PRSV). All the genotypes exhibited varying degrees of reaction against viral infection when categorized on 0-5 scale. The average Percentage Disease Severity (PDS) estimates ranged from 0.00 to 86.67. Out of the 33 genotypes, 3 were resistant, which went on asymptomatic all through the experiment. Four genotypes were resistant, five were moderately resistant, six were moderately susceptible, 10 were susceptible and five were highly susceptible. The resistant genotypes identified are promising candidates for crop improvement programmes to develop mosaic resistant cultivars in bitter gourd.

Keywords: Bitter gourd, *Momordica charantia*, resistance, Mosaic, Per cent Disease Severity.

INTRODUCTION

Bitter gourd often known as bitter melon (*Momordica charantia* L.) is one of Kerala's major cucurbitaceous vegetable crops. Bitter gourd is thought to have first appeared in the Old World tropics, specifically in eastern India and southern China (Garrison, 1977). Since bitter gourd flowering is monoecious in nature, it is a crop that is highly cross pollinated. The crop is in a diploid state with chromosome number 2n= 22. The fruits have a great nutritional and therapeutic value and are utilised in a range of culinary recipes. The fruits contain alkaloid compounds with potential medical value such as momordicine, saponine, and albuminoides and are high in vitamin C and folate. In addition to being used for consumption, fruits are said to have a variety of pharmacological qualities, including antioxidants, anti-diabetes, antibacterial, anti-cancer, and others (Fuangchan *et al*., 2011).

However, many biotic and abiotic constraints affect bitter gourd commercial production, including a severe threat from viruses. When plants are infected by viruses, they trigger intricate defense mechanisms that function at various levels, often at a significant energy cost to the plant, resulting in losses (Syller and Grupa 2016). Mosaic disease is becoming a major limiting factor for the cultivation of the crop, especially during summer season. Almost 35 different viruses have been isolated from cucurbitaceous vegetables (Provident, 1996). The important viruses affecting bitter gourd in Kerala are, *Cucumber mosaic virus* (CMV) (Nagarajan and Ramakrishnan 1971; Akbar *et al*., 2015), *Papaya ring spot 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). These viruses occur in complex or which may cause sole infection (Nameth *et al*., 1986). Symptoms of the virus infection include yellow specks, yellow mosaic, reduction in leaf size, short internodes, leaf curling, thickened leaf margins, and darkening, puckering, and severe stunting of the entire plant (Nagendran *et al.,* 2017; Gomathi *et al.,* 2023). In spite of the economic importance of this vegetable, the research work carried out on protection of crop from viral disease is quite scanty. Viruses are obligate intracellular parasites and till now no therapeutic treatments have been reported making it very difficult for the field management of the disease (Nicaise, 2014). Most of the high yielding varieties are being affected by this disease. The disease is mainly transmitted by insect

pests like aphids and whiteflies. Once the plant is infected with the virus, the only possible management measure is to control the vectors to prevent further disease spread. This has led to an unbiased application of insecticides causing health and environmental hazards. Hence healthy planting materials and disease resistant cultivars are essential for cultivation of bitter gourd with higher yield and good quality. Using a combination of resistant crop varieties and an integrated disease management system that is customized to different agro-ecological conditions can be a better and more ecologically friendly solution for addressing viral epidemics, even though cultural practices may also provide some benefit (Martín-Hernández and Picó, 2021; Yonchev and Dyulgerski 2021).

Considering the above mentioned facts, the current study was proposed with the main objective of screening the available bitter gourd germplasm to identify mosaic resistant sources, which can be further utilized in crop improvement programmes.

MATERIALS AND METHODS

A. Germplasm collection

Thirty three genotypes collected from National Bureau of Plant Genetic Resources (NBPGR), farmer's fields of various districts of Kerala and other states, popular high yielding varieties of KAU *viz.*, Preethi and Priyanka formed the material for the experiment. The study was conducted at Farming Systems Research Station, Sadananadapuram, Kottarakara, Kerala. The genotypes included both *M. Charantia var. charantia* and *M. charantia* var. *muricata,* which are included in the Table 1. Experiment was in Completely Randomized Design with six replications during June 2021– September 2021.

Table 1: List of genotypes used in the study.

No.	Genotype	Source
T1	Saidabad local	Saidabad
T2	JP Nagar local	JP Nagar
T3	Ariadaha local	Kolkata
T4	Lodhi local	New Delhi
T5	Onkar Nagar local	New Delhi
T6	Vellayani local	Vellayani
T7	Telangana local	Telangana
T8	Jaya nagar local	Bangalore
T9	Vyasarpadi local	Tamil Nadu
T10	Andhra Pradesh local	Andhra Pradesh
T11	Bangalore local	Bangalore
T12	Palappur local	Trivandrum
T13	Udayagiri local	Udayagiri
T14	Idukki local	Idukki
T15	Preethi	Kerala Agricultural University
T16	Priyanka	Kerala Agricultural University
T17	Alacode local	Alacode
T18	Therthali local	Therthali
T19	Iritty local	Iritty
T20	Thrissur local	Thrissur
T21	IC 85636	National Bureau of Plant Genetic Resources, New Delhi
T22	IC 45346	National Bureau of Plant Genetic Resources, New Delhi
T23	IC 44413	National Bureau of Plant Genetic Resources, New Delhi
T24	IC 68335	National Bureau of Plant Genetic Resources, New Delhi
T25	IC 50527	National Bureau of Plant Genetic Resources, New Delhi
T26	IC 596980	National Bureau of Plant Genetic Resources, New Delhi
T27	IC 68309	National Bureau of Plant Genetic Resources, New Delhi
T28	IC 113875	National Bureau of Plant Genetic Resources, New Delhi
T29	IC 68272	National Bureau of Plant Genetic Resources, New Delhi
T30	IC 68275	National Bureau of Plant Genetic Resources, New Delhi
T31	IC 85634	National Bureau of Plant Genetic Resources, New Delhi
T32	IC 85626	National Bureau of Plant Genetic Resources, New Delhi
T33	IC 33275	National Bureau of Plant Genetic Resources, New Delhi

B. Screening of genotypes under artificial inoculation with viruses

Wedge grafting was done to transmit viruses to healthy plants. At the time of grafting, infected twigs of bitter gourd were collected from the field and used as scions. Collected thirty three bitter gourd genotypes at 3-4 leaf stage were used as root stocks. Wedge or 'V' shaped cut was made on root stock, just one centimetre above cotyledonary leaf to insert the infected scion. Scion was prepared by making a tapering cut at the end so as to fix into the cut end of the rootstock. The graft union was joined using a graft clip. The grafted plants were protected by covering with clear plastic bag. The young shoots that emerged from the axil of the cotyledonary leaves of root stock were observed for the expression of symptoms.

C. Disease scoring

Expressions of mosaic symptoms (Fig. 1) were scored according to the 0-5 scale developed by Arunachalam *et al.* (2002)

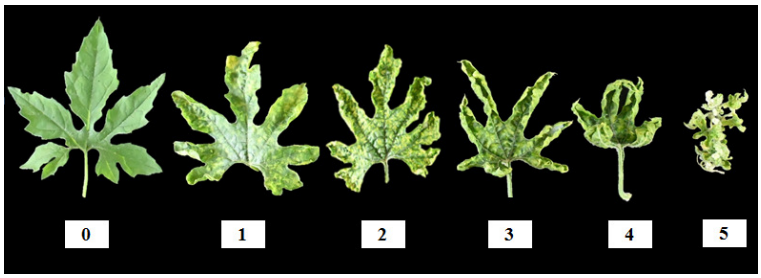

Fig. 1. Expression of mosaic symptoms in bitter gourd.

0 - No symptom
1 - Minute chlorotic/mosaic specks on leaf
2 - Wide area of mosaic symptoms on whole leaf without distortion
3 - Mosaic symptom with reduction of about 25 per cent of the normal leaf
4 - Mosaic symptom with reduction of about 25 to 75 per cent of the normal leaf
5 - Mosaic symptom with reduction of more than 75 per cent of the normal leaf area

Based on the scoring, Per cent Disease Severity (PDS) was found out with the formula,

$$\text{Percent Disease Severity (PDS)} = \left[\frac{[0n0 + 1n1 + 2n2 + 3n3 + 4n4 + 5n5]}{(\text{Total number of leaves observed} \times \text{Maximum disease grade})} \right] \times 100$$

Where, n0, n1, n2…n5 is the number of plants in score 0, 1,2…5

Based on PDS values, genotypes were grouped into six categories (Table 2).

Symptom development was scored on an individual plant basis 60 days after inoculation and all plants were evaluated for the presence of three viruses.

Table 2: Category of disease reaction based on PDS value (Arunachalam *et al.* 2002).

Disease severity (%)	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)

D. DAS-ELISA for detection of viruses

Double Antibody Sandwich ELISA was performed to know the presence or absence of three viruses namely, *Cucumber Mosaic Virus, Tomato leaf curl New Delhi virus* and *Papaya Ringspot Virus* in the inoculated bitter gourd plants. Procedure described by DSMZ Plant virus department, Braunschweig, Germany was followed for the detection.

After recommended dilution 200µl of diluted antibody was added to each well of the microtiter plate. Covered the plates with aluminium foil and kept for incubation at 37°C for 2- 4 h. After incubation washed the plate with PBS-Tween using ELISA plate washer (pw-40, BIORAD) soaked for a few minutes and repeated washing two times. Plates were botted by tapping upside down on tissue paper. Sample was extracted by homogenizing 1g of leaf sample in 5 ml of sample extraction buffer. For CMV and PRSV, PBS-Tween containing 2% PVP (pH 7.4) was used as sample extraction buffer where as for ToLCNDV, sample extraction was done using 0.05 M Tris containing 0.06 M sodium sulfite, (pH 8.5). The homogenate was centrifuged at 10000 rpm for 15 min at 4°C. Added 200 µl aliquot into wells of washed ELISA plate and replicated twice. Plate was covered again and incubated for overnight at 4°C. After incubation plate was washed three times in an ELISA plate washer as before. 200 µl enzyme conjugate was added to each well after recommended dilution in conjugate buffer. Plate was covered using aluminium foil and incubated for 2-4 h at 37°C. Washing with PBS-T using ELISA plate washer was repeated after incubation. 200 µl aliquots of freshly prepared substrate (1 mg /ml para- nitrophenyl-phosphate in substrate buffer) was added to each well. Covered the plate and incubated at 37°C for 30-60 min, or as long as necessary to obtain clear reactions. The absorbance was read at 405 nm in an ELISA reader (Microplate reader 680, BIORAD).

Samples were rated as infected when yellow colour developed in ELISA plates and its optical density (OD) value exceeded twice that of the negative control.

RESULTS AND DISCUSSION

A. Symptomology

The major viruses causing bitter gourd mosaic in Kerala such as *Cucumber Mosaic Virus (*CMV*), Papaya Ringspot Virus* (PRSV) and *Tomato Leaf Curl New Delhi Virus* (ToLCNDV) produced mixed infection in the field (Tiwari *et al*., 2010). The symptoms primarily manifested on the leaves located in the secondary branches at the top of the plant. The initial signs of the disease were small, irregular yellow patches on a limited number of leaves. In severe cases, the disease progressed to clear the veins in one or two sections of the leaf, and heavily affected plants displayed a decrease in leaf size, elongation, or suppression in one or two sections. Newly developing leaves were distorted and smaller in size. Some leaves exhibited a reduction in lamina development, resulting in a shoestring-like appearance.

Some accessions expressed typical symptoms of *Cucumber mosaic virus* (CMV) such as vein clearing, downward rolling of leaf margins, and a leathery appearance, which were described by Nagarajan and Ramakrishnan (1971). Meanwhile, symptoms like vein clearing, thin leaves, reduced leaf size, and yellowing, were observed in most accessions, noted by Ashwini (2015) as typical symptoms of *Poty virus*. Furthermore, symptoms of *Tomato Leaf Curl New Delhi Virus*

(ToLCNDV) infection were also present in some leaves of the plant. These symptoms initially appeared as small yellow spots on the outer edges of leaves, which quickly spread throughout the whole leaf, causing distortion and a reduction in size. These diseases produce characteristics symptoms such as mosaic mottling of leaves, reduced leaf size, crinkling of leaves, distortion of leaves, yellow vein, enation on lower side of leaves, stunted growth of plant, yellow mosaic patches on leaves, *etc*. Incidence in several instances recorded upto 100% yield loss (Kiran *et al*., 2021; Nagendran, 2017).

B. Reaction of genotypes to viruses

The susceptible and resistant groups comprising 33 genotypes were classified based on the calculated PDS of an accession at 105 DAS (Table 3).

The average Percentage Disease Severity (PDS) estimates ranged from 0.00 to 86.67%. Previous studies by Arunachalam *et al.* (2002) have also reported a PDS value that ranges from 0-76. Out of the thirty three genotypes screened for mosaic incidence, three were found to be highly resistant with a PDS value of zero (T4, T13, T18) which means that they had a lower percentage of disease severity compared to the other genotypes. Among that "T13 and T18 are wild bitter gourd genotypes of the *Momordica charantia* var. *muricata* species. Four genotypes (T1, T7, T17, T20) were found to be resistant with PDS ranged from 6.67 to 10.00. Five genotypes (T9, T10, T19, T21, T31) were moderately resistant with a PDS value ranging from 13.33 to 20.00. Genotypes T3, T8, T11, T14, T25 and T32 were moderately susceptible and ten of them (T2, T5, T6, T24, T12, T27, T28, T29, T30, T33) were susceptible. For moderately susceptible genotypes PDS value ranged from 23.33 to 36.67 whereas for susceptible ones the range extends from 43.33 to 70.00. Highly susceptible genotypes with severe mosaic symptoms observed are T15, T16, T22, T23 and T26, where PDS value reached up to 86.67.

Asna *et al.* (2018) conducted a study on 53 different accessions of bitter gourd to determine their level of resistance to a viral disease. The study revealed that none of the genotypes exhibited complete immunity to the disease. However, the accessions varied in their level of resistance, with five being classified as resistant, nine as moderately resistant, 25 as moderately susceptible, 11 as susceptible, and two as highly susceptible. In the current study, the Priyanka and Preethi varieties of KAU were found to be highly susceptible, while in earlier studies conducted by Asna *et al*. (2018); Ashwini (2015), they were reported to be susceptible only. Results of the experiment by Resmi and Sreelathakumary (2017); Radhika (2017) also supported the current findings. The current findings imply that the genotypes that are resistant to viral infection should be preserved for further research to confirm the causes of resistance by molecular detection and can be utilized for genetic improvement

C. Serological detection of viruses

The presence of viruses in highly resistant plants was detected using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

The genotypes, Lodhi local (T4), Udayagiri local (T13) and Therthali local (T18) did not exhibit any characteristic symptoms of three viruses even after inoculation. The genotype Udayagiri local showed the lowest levels of absorbance for CMV (0.005) and PRSV (0.003), while the Lodhi local exhibited least absorbance for ToLCNDV (0.006). DAS-ELISA results revealed that all the highly resistant genotypes recorded an ELISA absorbance value which was less than twice the absorbance value of un-inoculated healthy plants. These plants remained asymptomatic throughout the experiment due to their resistance mechanism. In a study conducted by Abdelkhalek *et al.* (2022), the presence of CMV was confirmed in squash plants using DAS-ELISA. Meanwhile, in a previous study by Yazdani-Khameneh *et al*. (2016), DAS-ELISA was utilized to detect the presence of ToLCNDV in cucurbits. Gomathi Devi (2022) reported that DAS ELISA conducted for whole seeds of bitter gourd recorded the OD value that ranged from 0.24 to 1.50 in embryo as compared to negative control (0.54). The presence of virus in the embryo is the major factor that decide the successful transmission of the begomoviruses to the off springs. In snake gourd the OD value for infected samples was recorded 0.566 with PRSV antiserum where as the negative control recorded OD value of 0.204 (Kumar *et al*., 2014).

Fig. 2. Genotypes resistant to mosaic incidence.

Table 3: Classification of genotypes based on PDS value.

Genotype	PDS Value (%)	Disease reaction
T1	10.00	R
T2	46.67	S
T3	30.00	MS
T4	0.00	HR
T5	63.33	S
T6	46.67	S
T7	10.00	R
T8	26.67	MS
T9	16.67	MR
T10	20.00	MR
T11	36.67	MS
T12	60.00	S
T13	0.00	HR
T14	23.33	MS
T15	73.33	HS
T16	86.67	HS
T17	6.67	R
T18	0.00	HR
T19	13.33	MR
T20	6.67	R
T21	20.00	MR
T22	80.00	HS
T23	76.66	HS
T24	56.66	S
T25	23.33	MS
T26	83.33	HS
T27	43.33	S
T28	66.67	S
T29	50.00	S
T30	70.00	S
T31	13.33	MR
T32	33.33	MS
T33	53.33	S

Table 4: ELISA values for the highly resistant genotypes.

Sr. No.	Genotype	ELISA Absorbance value		
		CMV	PRSV	ToLCNDV
1	Lodhi local	0.009	0.007	0.006
2	Udayagiri local	0.005	0.003	0.013
3	Therthali local	0.011	0.015	0.017
	Un-inoculated healthy plants	0.008	0.010	0.012

CONCLUSIONS

The current study was conducted to identify bitter gourd genotypes that are resistant to three viruses namely *Cucumber Mosaic Virus (*CMV*), Papaya Ringspot Virus* (PRSV) and *Tomato Leaf Curl New Delhi Virus* (ToLCNDV). Reaction of genotypes to viruses differed among the 33 genotypes and based on the disease reaction they were classified into six categories. Three highly resistant genotypes *ie*., Lodhi local, Udayagiri local and Therthali local were identified from the experiment. Presence of these viruses in the resistant genotypes was confirmed using DAS-ELISA.

FUTURE SCOPE

Despite the significant occurrence rate of mosaic virus in bitter gourd, limited resources are available to combat the disease. Consequently, there has been a recent increase in efforts to identify natural sources of resistance and resistance genes for efficient control of viruses affecting various vegetable crops globally. This study shows that all cultivated bitter gourd varieties are susceptible to the disease, but the genotypes identified as highly resistant can be tested for stability across different locations. These resistant genotypes can serve as source material for improving bitter gourd varieties against mosaic viruses. Additionally, studying disease development and varietal reaction will provide a base for future research in this area.

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Conflict of interests. None.

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