

# Identification of bitter gourd genotypes with field tolerance to viral diseases

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Received 22 May 2018; received in revised form 4 June 2018; accepted 21 June 2018

## Abstract

Diseases caused by viruses are a major problem in bitter gourd throughout India, causing considerable yield loss. Use of host plant resistance is the cheapest and effective disease management strategy. In the present investigation 50 genotypes of *Momordica charantia* var. *muricata* along with 3 susceptible check varieties viz., Preethi, Priyanka and Pusa purvi were screened against viral infections under natural epiphytotic conditions during the years 2016 and 2017. Both per cent disease severity (PDS) and area under disease progress curve (AUDPC) were calculated to assess and categorize the disease resistance in bitter gourd. They showed varying degrees of response against viral infection when categorized on 0-5 scale. Among 53 accessions evaluated, five were found resistant, nine moderately resistant, 25 moderately susceptible, 11 susceptible and two highly susceptible. None of the genotypes recorded immune response. The resistant genotypes can be utilized in breeding programme to evolve resistant varieties.

**Keywords:** AUDPC, Bitter gourd, Mosaic, PDS, Resistance, Screening

## Introduction

Bitter gourd (*Momordica charantia* L.) is one of the most important cucurbitaceous vegetables, valued for its nutritional and medicinal properties. The genus *Momordica*, belonging to the family Cucurbitaceae, is a native of the Paleotropics (Robinson and Decker-Walters, 1997). Even though *Momordica* is one of the largest genera in that family, *M. charantia* is the only cultivated species in this particular genus, on which profound studies were done. It has been in use for centuries in the traditional system of medicine of several countries like India, China, Africa and Latin America. *M. charantia*, commonly known as bitter gourd, balsam pear, bitter melon, bitter cucumber and African cucumber consists of two botanical varieties viz., *M. charantia* var. *muricata*, a wild variety with small and round fruits having markedly sculptured seeds, and *M. charantia* var. *charantia*, which produces

large fusiform fruits having feebly sculptured seeds (Chakravarthy, 1990). The wild variety (*M. charantia* var. *muricata*) is considered as the progenitor of the cultivated *M. charantia* var. *charantia* (Degner, 1947).

The fruits of cultivated types (*M. charantia* var. *charantia*) are widely consumed and have wide commercial distribution in Asia. Even though cultivated bitter gourd is highly vulnerable to pest and disease problems, its wild/ semi-domesticated types are endowed with resistance/ tolerance to some of the common pests and diseases of cucurbits (Bharathi and John, 2013). The *muricata* types are even reported to have potent anti-oxidant and free radical scavenging activities (Wu and Ng, 2008).

Among the various diseases affecting the crop, viral diseases commonly called as mosaic diseases are a major problem worldwide, causing losses as high

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as 100 per cent (Ullman *et al.* 1991). Almost 35 different viruses have been isolated from Cucurbitaceae family (Provident, 1996). Among these, Cucumber mosaic virus, Poty virus and Bitter gourd distortion mosaic virus are important in Kerala because of their wide distribution, severity of symptoms and heavy losses caused to cultivated bitter gourd (Ashwini *et al.*, 2016). These viruses may occur in complex or may cause sole infection.

Favourable environment for both vector and virus, lack of awareness about viral diseases, and abundance of viral vectors result in severe epidemics of many of these viral diseases. Farmers mainly depend on chemicals to control these viruses. Even though, partial control of viral diseases can be achieved with the application of certain pesticides, chemical control of the insect vector is mostly ineffective for the disease control. It also causes environmental hazard and pose health risks to the farmers and consumers. Use of resistant variety is the best and most economical method of viral disease control. It is cheap, easy to adopt and also environment friendly. The identification of sources of resistance to viral diseases is essential for the development of such cultivars. Therefore, as an initiative towards mosaic resistance breeding, the germplasm lines of wild bitter gourd (*M. charantia* var. *muricata*) were systematically field screened to identify the resistant sources against viral diseases, and the results are reported herein.

## Materials and Methods

Fifty accessions of wild bitter gourd (*M. charantia* var. *muricata*) collected from the Regional Station of National Bureau of Plant Genetic Resources (NBPGR), Thrissur and farmer's fields of various districts of Kerala, Karnataka and Tamil Nadu formed the material for the study. The popular high yielding varieties of bitter gourd *viz.*, Preethi and Priyanka, and wild bitter gourd *viz.*, Pusa Purvi, were used as check varieties. The experiment was raised in augmented block design in the experimental plot of Instructional Farm, Kerala

Agricultural University, Thrissur, during two consecutive seasons *viz.*, September- December of 2016 and 2017, to screen the wild bitter gourd germplasm lines against the viral diseases under natural field conditions.

The collected accessions were planted in pits of size 60 x 60 x 30 cm. The spacing between both the pits and rows was 2 x 2 m. Five plants per accession were maintained. All the recommended agronomic practices as per KAU (KAU, 2007) were followed for raising a healthy crop; in addition, insecticides were not applied throughout the crop stand to ensure adequate vector population for viral infection in the field. Further, the susceptible variety Priyanka was raised all around the field to provide the disease inoculum facilitating screening of the entries under field conditions.

Symptomatology of viral diseases observed was recorded under natural field conditions. The viral disease incidence and severity were recorded from each plant at 60, 75, 90 and 105 days after sowing. Observations on disease reaction were made on all the plants in each entry. Twenty leaves were scored in each plant, ten from the apical region and five each from the middle and basal region, and all of them were graded. A modified 0-5 visual scale of Arunachalam *et al.* (2002) based on disease symptoms, was used to score the diseased plants as follows: 0- No symptoms; 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 (Plate 1). Based on the disease score, Per cent Disease Severity (PDS) was worked out as given below:  
Per cent Disease Severity (PDS) = [Sum of all numerical ratings/(total number of leaves observed x maximum disease grade)]100

Based on PDS values, genotypes were grouped into

Table 1. Categorization of genotypes based on PDS values

Disease severity (%)	Disease reaction
0.0 to 5.0	Highly Resistant (HR)
5.1 to 10.0	Resistant (R)
10.1 to 20.0	Moderately Resistant (MR)
20.1 to 40.0	Moderately Susceptible (MS)
40.1 to 70.0	Susceptible (S)
70.1 to 100.0	Highly Susceptible (HS)

\*Arunachalam et al., 2002

six categories (Table 1).

The areas of disease progress on the accessions or varieties were calculated using PDS values. The area under disease progress curve (AUDPC) for disease severity was calculated using the modified formula described by Shaner and Finney (1977) as below:  $AUDPC = \sum^{n-1} [(DS_1 + DS_2)/2] \times (t_2 - t_1)$  where,  $DS_1$  is disease severity recorded in time 1 ( $t_1$ ) and  $DS_2$  the

## Results and discussion

A set of 53 accessions of bitter melon comprising of germplasm accessions of wild bitter melon, and 3 susceptible check varieties were sown in augmented design under field conditions for the evaluation of their resistance against viral infection during the year 2016 and 2017. The level of resistance and susceptibility varied with the accessions.

After 50-60 days of sowing, the vector (white flies) started to appear in low densities and continued their build up during the whole growth period of bitter melon during 2016 and 2017. The occurrence of the first symptoms was approximately 50 to 60 days after sowing (DAS). The viral infection was mostly

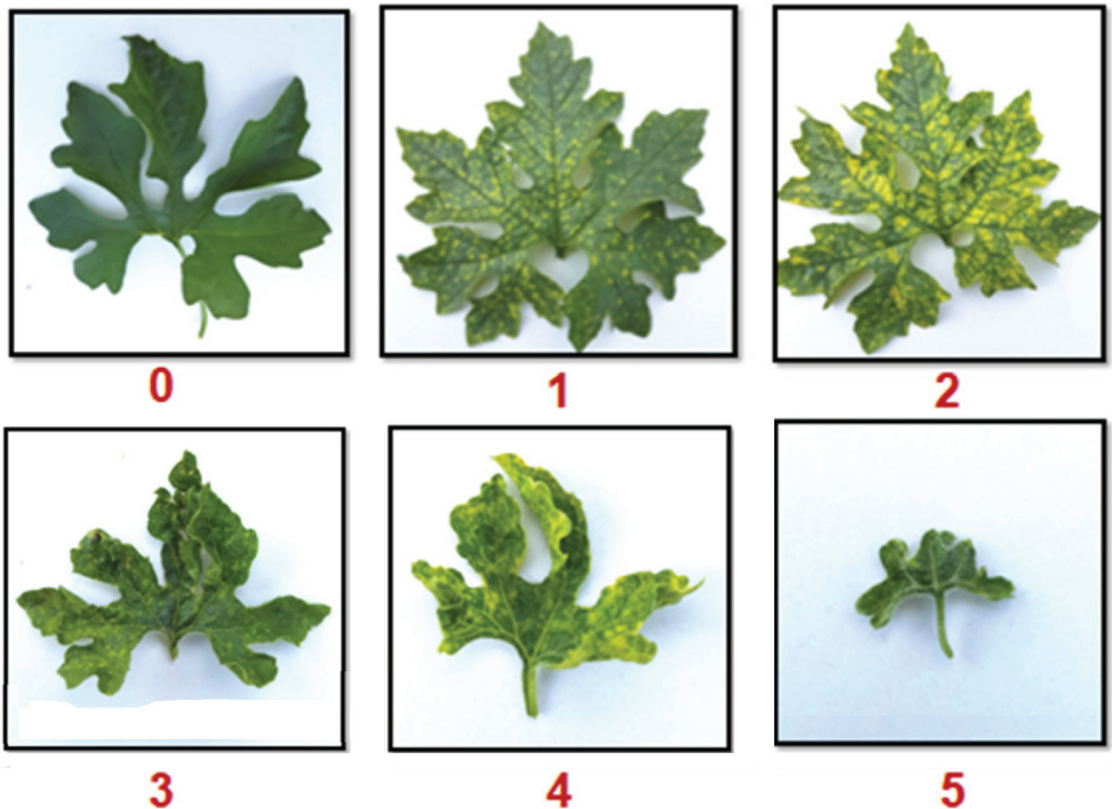


Plate 1. 0-5 visual scales used to score the viral disease infection

\*0=no symptoms, 1= Minute chlorotic specks/ patches on leaf, 2= Wide area of mosaic symptoms on whole leaf without distortion, 3= Mosaic symptoms with 1-25% reduction of leaf area, 4= Mosaic symptom with 25-75% reduction of leaf area, 5= Mosaic symptom with more than 75% reduction of leaf area

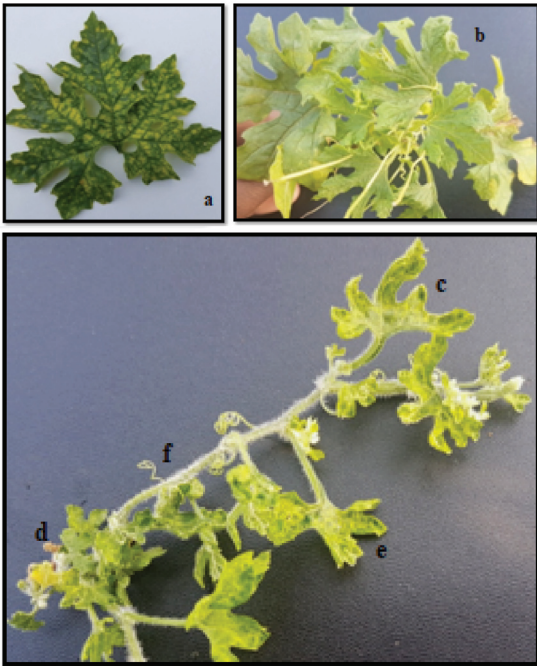


Plate 2. Viral disease symptoms appeared in the field under natural condition

a-yellowing; b- clustering of internodes and leathery leaf with marginal leaf rolling; c- cupping; d- reduced leaf size; e- mosaic and puckering; f- hairy growth

confined to the leaves and exhibited typical symptoms such as vein clearing, mosaic, mottling, vein banding, and leaf malformation. The wild bitter melon accessions under study displayed a wide range of disease symptoms (Plate 2). The symptoms mainly appeared on the leaves in the secondary branches produced at the apical end of the plant. The first disease symptoms initiated as small irregular yellowish patches on a few leaves of a small number of plants. Severe symptoms started with vein clearing in one or two lobes of the leaf and severely infected plants showed reduction in leaf size and elongation and/or suppression of one or two lobes. Young developing leaves were completely distorted and malformed with considerable reduction in their size. Some of the leaves showed marked reduction in the development of lamina resulting in a shoestring effect.

Bitter melon (*M. charantia*) has been reported to be

the natural host of many viruses (Tiwari *et al.*, 2010) like Cucumber Mosaic Virus (CMV), Poty virus and Bitter melon Distortion Mosaic Virus (BDMV) (Ashwini *et al.*, 2016). The major viruses causing bitter melon mosaic in Kerala like CMV, Poty virus and BDMV produced mixed infection in the field. The simultaneous occurrence of different viruses in bitter melon plants resulted in the development of mosaic complex.

In some accessions, typical symptoms of CMV like vein clearing, downward rolling of leaf margin and leathery appearance was observed as described by Nagarajan and Ramakrishnan (1971). Symptoms like vein clearing, filiform leaves, reduced leaf size and yellowing, were present in most of the accessions, which were noted by Ashwini *et al.* (2016) as typical Poty virus symptoms. In addition to CMV and Poty virus, the symptoms of BDMV infection also appeared in some leaves of the plant. It first appeared in the newly formed leaves and rapidly spread to other leaves on the same vine. This symptom initially appeared as small chlorotic specks on the outer margins of leaves, which spread rapidly through the whole leaf. Later the infected leaves got distorted and reduced in size. In severe cases, enhanced hairy growth was observed on vines and leaves. Shortening of internodes and clustering of leaves were also observed. The early infection of BDMV led to stunted growth, clustering of leaves and sterility of plants. Mixed infections of all these viruses were observed in majority of the accessions. The accessions were initially scored for viral infection 60 days after sowing when the symptoms started appearing on the plants, using a modified visual scale of 0-5 (Table 1). Viral infection severity was estimated at 15 days intervals till there was no further significant disease prevalence. The accessions showed varying degrees of response against viral infection when categorized on 0-5 scale. The PDS values during 2016 and 2017 after 60, 75, 90 and 105 days of sowing were calculated and are given in Table 2.

The PDS values increased with the passage of time

*Table 2.* Disease severity and AUDPC values of bitter melon genotypes to viral infections under field conditions at 15 days interval during 2016 and 2017

Sl. No.	Accessions	PDS (2016) at				AUDPC (2016)	PDS (2017) at				AUDPC (2017)
		60	75	90	105		60	75	90	105	
		DAS	DAS	DAS	DAS		DAS	DAS	DAS	DAS	
1	JJNS-15/12	0.00	0.00	5.00	13.00	172.50	0.00	5.00	18.00	24.67	530.03
2	JJNS-15/39	0.00	0.00	8.00	17.00	247.50	0.00	2.67	11.00	16.33	327.53
3	JJNS-15/43	5.67	12.33	24.67	36.00	867.53	0.00	0.00	15.00	19.33	369.98
4	JJNS-15/62	7.00	18.67	31.00	44.67	1132.58	5.00	11.67	18.01	21.33	642.68
5	JJNS-15/65	0.00	0.00	0.00	6.00	45.00	0.00	0.00	0.00	7.33	54.98
6	IC 213312	0.00	0.00	2.67	9.67	112.58	0.00	0.00	0.00	5.00	37.50
7	IC 467681	0.00	0.00	0.00	8.00	60.00	8.33	11.00	17.33	20.67	642.45
8	W 84	5.00	11.67	27.33	34.00	877.50	10.01	18.67	26.00	30.67	975.15
9	IC 541231	5.00	8.67	31.00	39.00	925.05	11.00	15.33	36.00	42.67	1172.48
10	IC 541235	4.33	9.67	29.00	31.00	845.03	4.33	8.67	27.67	36.67	852.60
11	IC 541248	5.00	16.00	31.67	46.00	1097.55	9.33	12.67	39.00	44.00	1175.03
12	IC 467673	10.33	38.67	59.00	83.33	2167.50	13.00	17.33	50.00	69.33	1627.43
13	IC 467645	8.00	42.33	62.67	79.67	2232.53	12.01	23.00	50.67	68.00	1705.13
14	IC 541377	0.00	2.33	11.00	15.67	317.48	0.00	0.00	10.67	15.33	275.03
15	IC 582403	1.00	7.67	18.33	25.33	587.48	0.00	18.33	20.00	23.00	747.45
16	IC 582434	3.00	14.00	22.33	29.00	784.95	6.67	16.33	20.01	27.33	800.10
17	IC 582471	4.00	9.00	27.00	38.00	855.00	11.01	22.00	37.00	43.33	1292.55
18	IC 598168	5.67	21.33	39.67	45.67	1300.05	14.00	19.00	29.00	42.67	1145.03
19	IC 598170	5.00	18.67	27.00	34.67	982.58	9.01	20.00	35.00	40.67	1197.60
20	IC 598171	2.00	8.33	19.00	27.33	629.93	2.00	7.00	15.00	24.00	525.00
21	IC 598172	2.33	5.67	15.33	22.67	502.50	4.33	17.67	27.00	36.67	977.55
22	W 154	5.00	15.33	28.00	38.00	972.45	5.01	10.67	26.67	35.00	860.18
23	W 172	0.00	5.00	17.67	21.00	497.55	12.33	28.00	30.00	35.67	1230.00
24	W 173	0.00	4.00	10.00	18.67	350.03	0.00	2.00	5.00	11.00	187.50
25	AC-16/1	0.00	0.00	0.00	9.33	69.98	0.00	0.00	0.00	4.57	34.28
26	AC-16/2	0.00	10.33	15.67	22.00	555.00	15.00	29.00	37.33	52.33	1499.93
27	AC-16/3	0.00	0.00	0.00	5.01	37.58	0.00	5.00	19.00	37.00	637.5
28	AC-16/4	0.00	7.00	10.67	17.00	392.55	0.00	0.00	5.01	10.67	155.18
29	AC-16/5	5.00	19.00	32.33	45.00	1144.95	16.33	31.33	45.00	56.67	1692.45
30	AC-16/6	0.00	0.00	0.00	5.33	39.98	0.00	2.33	7.67	14.67	260.03
31	AC-16/7	6.00	22.33	39.67	45.67	1317.53	0.00	0.00	11.33	19.67	317.48
32	AC-16/8	0.00	17.00	19.00	24.33	722.48	0.00	14.67	20.33	24.33	707.48
33	AC-16/9	0.00	0.00	0.00	3.67	27.53	0.00	0.00	0.00	8.00	60.00
34	AC-16/10	0.00	0.00	0.00	8.67	65.03	0.00	5.67	10.67	16.33	367.58
35	AC-16/11	1.00	19.67	27.67	39.00	1010.10	4.01	18.67	22.33	39.67	942.60
36	AC-16/12	4.00	20.00	25.00	34.33	962.48	9.01	24.67	35.00	43.67	1290.15
37	AC-16/13	5.00	21.00	31.00	45.00	1155.00	2.67	18.67	20.00	27.00	802.58
38	AC-16/14	2.00	14.67	19.33	29.67	747.53	5.33	19.01	30.01	38.33	1062.75
39	AC-16/15	0.00	16.33	18.67	21.00	682.50	4.33	16.00	20.00	25.67	765.00
40	AC-16/16	0.00	11.00	20.00	25.33	654.98	10.01	15.00	29.00	30.33	962.55
41	AC-16/17	0.00	13.00	21.67	31.00	752.55	9.67	22.33	26.00	39.33	1092.45
42	AC-16/18	1.00	8.00	21.00	28.67	657.53	15.01	24.00	42.00	56.67	1527.60
43	AC-16/19	10.00	21.67	42.67	50.33	1417.58	6.67	27.33	30.00	38.00	1194.98
44	AC-16/20	0.00	0.00	5.00	12.67	170.03	6.67	20.67	30.01	36.67	1085.25
45	AC-16/21	0.00	0.00	0.00	9.67	72.53	0.00	0.00	0.00	10.67	80.03
46	AC-16/22	2.00	17.33	29.67	45.33	1059.98	0.00	0.00	18.67	28.33	492.53
47	AC-16/23	1.33	9.67	15.00	22.33	547.50	0.00	0.00	5.67	15.33	200.03
48	AC-16/24	2.00	10.00	33.67	40.00	970.05	0.00	25.00	40.33	43.00	1302.45
49	AC-16/25	3.00	18.00	26.00	34.67	942.53	3.33	15.01	20.00	25.67	742.65
50	AC-16/26	8.67	21.67	45.67	50.00	1450.13	11.00	23.67	48.01	51.67	1545.23
51	Preethi	10.67	24.00	39.33	48.00	1389.98	18.01	29.00	38.67	56.00	1570.13
52	Priyanka	12.00	28.00	47.33	56.01	1640.03	19.33	40.00	45.33	53.00	1822.43
53	Pusa purvi	0.00	5.00	20.00	28.67	590.03	0.00	24.00	29.67	49.67	1177.58

*Table 3.* Disease reaction of bitter gourd genotypes to viral infections under field conditions

Sl. No.	Accessions	PDS means	Symptoms	Disease reaction
1	JJNS-15/12	18.84	M, m, LC, Y	MR
2	JJNS-15/39	16.67	M, LC, N	MR
3	JJNS-15/43	27.67	m, Y, LC, N	MS
4	JJNS-15/62	33.00	M, m, N, Y	MS
5	JJNS-15/65	6.67	Y, M	R
6	IC 213312	7.34	M, m	R
7	IC 467681	14.34	Y, SL	MR
8	W 84	32.34	M, m, SL	MS
9	IC 541231	40.84	UC, M, m	S
10	IC 541235	33.84	VC, CL	MS
11	IC 541248	45.00	S, M, m	S
12	IC 467673	76.33	VC, Y, M, m, CL	HS
13	IC 467645	73.84	Y, SL, UC, M, m	HS
14	IC 541377	15.50	CL, S	MR
15	IC 582403	24.17	M, Y, SL	MS
16	IC 582434	28.17	VC, CL, S, M	MS
17	IC 582471	40.67	M, m, CL	S
18	IC 598168	44.17	UC, CL, N	S
19	IC 598170	37.67	N, M, m, Y	MS
20	IC 598171	25.67	Y, M, SL	MS
21	IC 598172	29.67	UC, VC, CL, Y	MS
22	W 154	36.50	Y, M, SL, m	MS
23	W 172	28.34	SL, VC, CL	MS
24	W 173	14.84	S, m, N	MR
25	AC-16/1	6.95	M, Y	R
26	AC-16/2	37.17	Y, m	MS
27	AC-16/3	3.51	Y	HR
28	AC-16/4	13.84	Y, SL, CL	MR
29	AC-16/5	50.84	m, VC, UC, Y	S
30	AC-16/6	10.00	Y, m	R
31	AC-16/7	32.67	M, m	MS
32	AC-16/8	24.33	Y, LC	MS
33	AC-16/9	5.84	Y, m, VC, LC	R
34	AC-16/10	12.50	LC, Y, M	MR
35	AC-16/11	39.34	M, Y, m	MS
36	AC-16/12	39.00	N, m, Y	MS
37	AC-16/13	36.00	Y, M	MS
38	AC-16/14	34.00	M, SL	MS
39	AC-16/15	23.34	Y, LC, VC, CL	MS
40	AC-16/16	27.83	Y, SL, N	MS
41	AC-16/17	35.17	M, VC, UC, CL	MS
42	AC-16/18	42.67	Y, LC, CL, SL	S
43	AC-16/19	44.17	Y, M, VC	S
44	AC-16/20	24.67	m, LC, Y	MS
45	AC-16/21	10.17	M, LC	MR
46	AC-16/22	36.83	M, Y, LC, VC	MS
47	AC-16/23	18.83	LC, CL, Y, M	MR
48	AC-16/24	41.50	M, m, LC, Y	S
49	AC-16/25	30.17	Y, LC, VC	MS
50	AC-16/26	50.84	M, m	S
51	Preethi	52.00	Y, LC, UC, M, m, N	S
52	Priyanka	54.51	LC, CL, Y, m, M	S
53	Pusa purvi	39.17	Y, m, M, N, S, UC	MS

M= mosaic, m=mottle, LC= leaf curling, VC= vein clearing, UC, upward curling, Y= yellowing, N= necrosis, CL= clustering

depending upon the genotypes. Plants infected at an early stage of growth exhibited severe symptoms, while mid season and late infections were milder and expressed reduced symptoms. The final PDS of an accession calculated at 105 DAS was the value considered for classifying 53 genotypes into the susceptible and resistant groups. PDS varied from 3.67 to 83.33% during 2016; and 4.57 to 69.33% during 2017 (Table 2). During 2016, the accession AC-16/9 (PDS- 3.67), was found to be highly resistant to viral infections. This accession showed a disease severity of 8 per cent during 2017 and was categorized under resistant group. During 2017 also, a single accession AC-16/1 (PDS-4.57), showed high resistance but its per cent disease severity was 9.33 during 2016. Thus the genotypes showing highly resistant to resistant response during one year became moderately susceptible to susceptible in the next year or vice versa.

So the PDS means were calculated using the PDS values of both seasons, noted 105 days after sowing. These mean values were later used to categorize the accessions into various disease severity groups (Table 3). Among 53 accessions scored, five were found resistant, nine moderately resistant, 25 moderately susceptible, 11 susceptible and two highly susceptible. The check varieties like Preethi and Priyanka were categorized as susceptible and wild bitter gourd variety Pusa Purvi was categorized as moderately susceptible.

The disease scoring done was used to get the gradual development of the disease and to calculate the area under disease progress curve (AUDPC). AUDPC was calculated between 60 and 105 DAS to estimate the magnitude of disease. The data are presented in Table 2.

The AUDPC is a useful quantitative summary of disease severity over time, for comparison across years, locations or management tactics. Disease severity in each accession at 2 different seasons was calculated and expressed as the AUDPC as shown in Table 2. During 2016, among 53 accessions

evaluated, four, viz., JJNS-15/65 (45.00), AC-16/3 (37.58), AC-16/6 (39.98), AC-16/9 (27.53) having AUDPC values less than 50.00, were found to show resistance against viral infections. Out of these 4 accessions, AC-16/9 was found to be highly resistant based on both PDS and AUDPC values. Two accessions, IC 467673 (2167.50) and IC 467645 (2232.53) were found to be highly susceptible. The AUDPC values of these highly susceptible accessions were even higher than that of the check varieties used for evaluation.

During 2017, the same set of genotypes was evaluated and the data are presented in Table 2. Among the genotypes evaluated, AC-16/1 belonging to wild bitter gourd exhibited the least AUDPC value (34.28). The highly susceptible reaction was shown by the check variety Priyanka with an AUDPC value of 1822.43.

Even though the AUDPC values of each accession varied with two years, the PDS and AUDPC values of respective accessions are comparable with respect to their disease reaction towards viral infections. Four accessions namely JJNS-15/65, AC-16/1, AC-16/9 and AC-16/21 developed symptoms of viral infection only 90 DAS during 2016 and 2017. These accessions showing late season symptom development showed a remarkable tolerance against viral infections and the yield loss was also low when compared to other accessions. So these accessions which showed resistant reaction against the viral diseases can be used in resistance breeding programmes.

The present findings suggest that the genotypes showing resistance to viral infection should be maintained for further studies for confirming the resistance sources under artificial conditions and for genetic manipulation.

### Acknowledgements

The first author is thankful to Director, NBPGR, New Delhi, India for providing the germplasm and

the Department of Plant Breeding & Genetics, COH, Vellanikkara, Thrissur for providing necessary facilities to conduct the experiment.

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