Journal of Applied and Natural Science
11(1): 199-204 (2019)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Biological confirmation of resistance from segregating populations of Gherkin (*Cucumis anguria* L.) against cucumber mosaic virus (CMV)

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Abstract
Cucumber Mosaic Virus (CMV) is most widespread and destructive disease of gherkin (*Cucumis anguria* L.) Most of the commercial varieties are susceptible to CMV disease. Thus, identification of resistant genotypes for management of CMV disease in gherkin is essential. A total of 179 F3 progenies derived from crosses of resistant and susceptible parent's *viz*., Acc.1 (susceptible) x Acc. 50 (resistant), Acc.3 (susceptible) x Acc.50 (resistant), Acc.48 (susceptible) x Acc 50 (resistant) were screened for CMV. Among 179 F3 families, 7 were Immune, 17 were Resistant, 76 were Moderately Resistant, 73 were Moderately Susceptible and 6 were Susceptible. The immune and resistant progenies were further confirmed for their resistance reaction by aphid transmission. Significant difference between the estimates of PDI or F3 progenies mapped into different response classes justified the classification.

Keywords: CMV, F3 Progenies, Gherkin, Resistant, Screening, Susceptible

Article Info
DOI: 10.31018/jans.v11i1.1979
Received: December 27, 2018
Revised: February 26, 2019
Accepted: February 28, 2019

How to Cite
Venkatesh, H.L. *et al.* (2019). Biological confirmation of resistance from segregating populations of Gherkin (*Cucumis anguria* L.) against cucumber mosaic virus (CMV). *Journal of Applied and Natural Science*, 11(1): 199-204

INTRODUCTION

Cucurbits, which belong to the family *Cucurbitaceae* are important vegetable crops which include melons, squash, cucumber, gourds, pumpkin and gherkin. Cucurbits are good sources of carbohydrates, vitamin A and C and minerals (Nath, 1979). Among cucurbits, gherkin (*Cucumis anguria* L.) also known as pickling cucumber was introduced to India in 1990. It is one of the important export oriented crops commonly known as West Indian burr gherkin. Among gherkin growers, it is commonly known as small cucumber. In India, gherkin is a 100 per cent export oriented crop as there is very little scope for domestic consumption. The export of gherkins is valued upto Rs.502 crores annually. Bottled gherkins pickled in vinegar contribute nearly 50 per cent of the exports (Sukumaran, 2007). Since, gherkin is a high value export oriented crop and area planted to gherkin is increasing rapidly. Consequently, gherkin is being grown on a variety of soils under varied agro-climatic situations. As a result, the crop has become susceptible to many biotic and abiotic stress. Gherkin is susceptible to a variety of fungal and viral diseases (Mugadur and Nittur, 2011). Of these, diseases caused by viruses constitute the major biotic constraints in gherkin production. The important viral diseases includes those caused by Cucumber Mosaic Virus (CMV), Zucchini Yellow Mosaic Virus (ZYMV), Papaya Ring Spot Virus (PRSV), Watermelon Mosaic Virus (WMV), Potato Virus Y (PVY), Tobacco Mosaic Virus (TMV), Tobacco streak virus (TSV) and Tomato Spotted Wilt Virus (Tospo virus) (Gracia, 2000; Krishna Reddy *et al.,* 2003; Viraktamath *et al*., 2003; Mugadur and Nittur, 2011).

Among virus diseases, Cucumber mosaic virus (CMV) is most destructive causing yield losses as

high as 40–60 per cent (Varma and Giri, 1998). CMV, a positive-sense ssRNA plant virus with a tripartite genome, is the type member of the genus C*ucumovirus* in the family *Bromoviridae* (Ribicki, 1995). The CMV is transmitted by *Aphis gossypii* Glover and *Myzus persicae* in a non-persistent manner (Chandankar *et al.*, 2013; Coudriet, 1962). Most of the commercial varieties of Gherkins are susceptible to CMV and the currently available chemical/cultural methods are either ineffective or uneconomical. Thus, identification of resistant genotypes for management of CMV disease in gherkin (*Cucumis anguria* L.) is essential. The present study was to identify and select progenies resistance to CMV from F3 segregating populations.

MATERIALS AND METHODS

Plant material: The gherkin material (*Cucumis anguria* L.) consisted of 179 F3 progenies derived from crosses of resistant and susceptible parent's *viz*., Acc.1 (susceptible) x Acc. 50 (resistant), Acc.3 (susceptible) x Acc.50 (resistant), Acc.48 (susceptible) x Acc 50 (resistant). The crosses Acc. 1 x Acc. 50, Acc. 3 x Acc. 50 and Acc. 48 x Acc. 50 will be hereafter referred to as A 1-50, A 3 -50 and A 48-50, respectively.

Preparation of inoculum: Young leaves of susceptible check showing typical symptoms of CMV were grounded in a pestle and mortar with 0.1 M phosphate buffer (pH 7.0) containing 0.2 % sodium sulfite in the ratio of 1:5 (g:ml) leaf and buffer. The sap was then filtered through a double layered muslin cloth and collected in a beaker. About 1.0 % of celite 545 was added to the sap as an abrasive. This inoculum was applied with a cotton swab on the young leaves of 10-15 days old test plants. After inoculation, the inoculated plants were lightly misted with distilled water and maintained in the insect proof greenhouse for symptom expression. Then per cent disease index was calculated.

Screening F3 progenies for response to CMV disease: Thirty seeds of 179 F3 progenies were sown in ten polythene covers of 4' × 6' with three seeds per polythene cover filled with a mixture of soil, manure and coir pith. An equal number of seeds of susceptible check were sown in similar manner as a positive control. The 12 to 15 days old seedlings of F3 progenies along with susceptible check were inoculated with CMV by sap inoculation method as described by Mandal *et al.* (2001) under glass house condition at Main Research Station (MRS), Hebbal, Bengaluru.

Disease scoring and estimation of *Per cent* Disease Index (PDI): The seedlings of F3 progenies were scored using 0-5 scale (Bos, 1982) for the disease based on the symptom typical to the disease at 10-days interval for a period of 30 days after inoculation (DAI).

Scale used to score symptoms typical to CMV disease

Scale		Description of symptoms
0	:	No symptoms
1	:	Very light mottling of older leaves and dark green colour in younger leaves
2	:	Light and dark green areas associated with veins
3	:	Mosaic, blistering and puckering of leaves
4	:	Distortion of leaves
5	:	Stunting of the plants with negligible or no flowering

Per cent disease index (PDI) was calculated by using the following formula (Silbernagel and Jafari, 1974).

$$PDI = 0n_0+1n_1+2n_2+3n_3+4n_4+5n_5 \text{ / nt (nC-1) X } 100 \quad \ldots\ldots\ldots Eq.1$$

Where, n_0, n_1, n_2, .., n_5 = No. of plants in score 0, 1, 2,.., 5, respectively, nt= total no. of plants, and nC= Total number of categories.

Classification of F3 progenies into different response groups based on estimates to PDI (Havey, 1996):

Estimate of PDI	Disease reaction
0	: Immune (I)
1-25	: Resistant (R)
26-50	: Moderately resistant (MR)
51-75	: Moderately susceptible (MS)
76-100	: Susceptible (MS)

The significance of difference in mean PDI of F3 progenies classified into different groups was pre-examined using F test.

Screening of selected F3 progenies by Aphid Transmission

Maintenance of aphid culture: Aphid species *Aphis gossypii* Glover was multiplied on cotton plants. The cotton plants were kept inside small insect proof wooden cages, previously sprayed with Dimethoate 0.2 per cent to ensure insect free condition of cages (Plate 1).

Pre-acquisition period (Starvation): Aphids were allowed to starve for 2 hr. in a petri dish, placed in a dark chamber before releasing on to the symptomatic leaves of infected cotton plants for acquisition feeding.

Acquisition access period (AAP): The pre-starved aphids were allowed to feed on infected gherkin leaves showing characteristic mosaic symptoms and were kept turgid by putting a cotton swab at the detached end of the leaf petiole. The aphids were allowed for acquisition feeding time of 10min. After acquisition feeding period the viruliferous aphids were released on to gherkin seedlings of selected F3 progenies at the rate of 10 aphids per genotype. After 10 min. of IAP, aphids were killed by spraying 0.05 *per cent* Imidacloprid. The inoculated plants were kept in insect proof cages for symptom development.

RESULTS AND DISCUSSION

Response of F3 progenies for CMV disease infection: The present study showed that PDI of Gherkin (*Cucumis anguria* L.) 179 F3 progenies ranged from 0% to 86.67%. Based on mean PDI

Table 1. Grouping of gherkin F3 progenies based on disease reaction to Cucumber mosaic virus.

PDI (%)	Disease reaction	Families derived from crosses		
		Acc.1 x Acc. 50	Acc.3 x Acc. 50	Acc.48 x Acc. 50
0	Immune (07)	17, 35 and 62	26 and 34	11 and 47
1-25	Resistant (17)	65	5, 8, 16, 18, 22, 38 and 43	24, 31, 40, 43,48, 60, 61, 66 and 67
26-50	Moderately resistant (76)	2, 6, 7, 8, 11, 13, 26, 27, 42, 44, 50, 51, 54, 55, 56, 57, 58, 60, 64, 66 and 67	4, 8, 9, 10, 13, 14, 15, 17, 21, 23, 24, 25, 27, 28, 29, 31, 32, 37, 37, 39, 39, 40, 42, 44, 45, 46, 50 and 52	2, 5, 12, 14, 16, 17, 18, 22, 25, 27, 34, 36, 38, 39, 41, 42, 44, 46, 50, 51, 52, 55, 56, 58, 63, 64, 72
51-75	Moderately susceptible (73)	1, 3, 4, 5, 10, 14, 15, 19, 21, 22, 23,24, 25, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41, 43, 45, 46, 49, 52, 53, 60 and 63	1, 2, 6, 11, 12, 20, 33, 35, 41, 47, 48 and 51	1, 5, 6, 8, 9, 10, 13, 15, 19, 20,23, 26, 29, 30, 32, 33, 35, 37, 49, 53, 54, 62, 65, 68, 69, 70 and 74
76-100	Susceptible (06)	20	3, 19 and 30	4 and 57

Table 2. Mean PDI of F_3 families classified into different response groups.

Disease reaction categories	Acc. 1-50		Acc. 3-50		Acc. 48-50	
	Number of F3 families	Mean PDI	Number of F3 families	Mean PDI	Number of F3 families	Mean PDI
Immune	03	00.00	02	00.00	02	00.00
Resistant	01	14.67	07	13.38	09	19.27
Moderately resistant	21	40.18	28	39.68	27	39.97
Moderately susceptible	34	62.04	12	62.28	27	58.93
Susceptible	01	84.00	03	83.33	02	85.00
F value	98.50		94.61		134.26	
P value	2.11E-24		7.19E-22		8.36E-30	

PDI- Per cent disease incidence

Table 3. F3 progenies with immune and resistant response to CMV disease infection under challenged disease pressure.

Category of Disease reaction	Families	Per cent Disease Index (PDI)			Mean PDI
		10 DAI	20 DAI	30 DAI	
Immune (Seven families)	1-17	0.00	0.00	0.00	0.00
	1-35	0.00	0.00	0.00	0.00
	1-62	0.00	0.00	0.00	0.00
	2-36	0.00	0.00	0.00	0.00
	2-34	0.00	0.00	0.00	0.00
	3-11	0.00	0.00	0.00	0.00
	3-47	0.00	0.00	0.00	0.00
Resistant (17 families)	1-65	0.00	24.72	42.86	22.86
	2-5	0.00	14.67	19.05	11.24
	2-8	0.00	8.56	21..08	23.93
	2-16	0.00	12.67	21.12	9.89
	2-18	0.00	14.98	23.48	12.82
	2-22	0.00	0.00	34.28	11.43
	2-38	0.00	11.12	26.78	12.63
	2-43	0.00	10.68	26.67	11.68
	3-24	0.00	5.61	36.00	13.87
	3-31	0.00	13.36	25.72	13.03
	3-40	0.00	15.47	18.78	11.42
	3-43	0.00	12.48	26.75	13.08
	3-48	0.00	13.69	19.27	10.96
	3-60	0.00	9.98	21.21	10.39
	3-61	0.00	14..44	23.33	12.59
	3-66	0.00	14.67	19.56	11.41
	3-67	0.00	10.68	21.33	10.67

1= F_3 families derived from cross Acc.1 x Acc. 50; 2= F_3 families derived from cross Acc.3 x Acc. 50;3= F_3 families derived from cross Acc.48 x Acc. 50

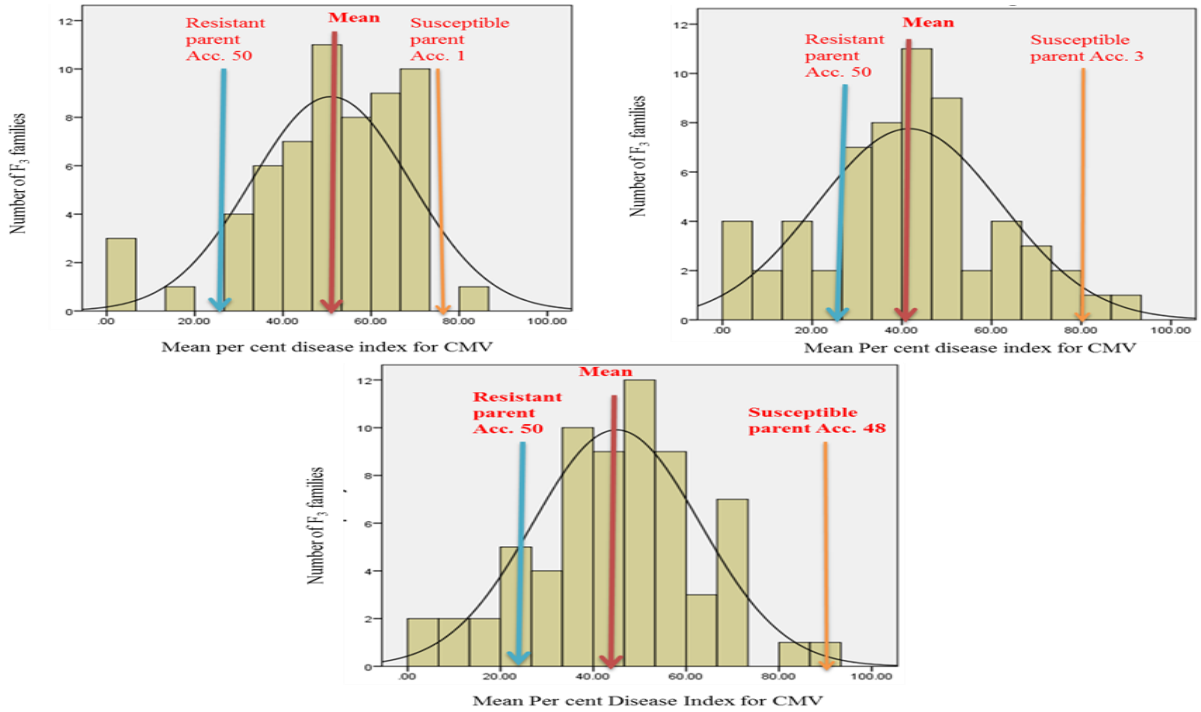

Fig. 1. *Distribution of F_3 families derived from the Acc. 1 ×Acc. 50, Acc. 3 x Acc. 50 and Acc. 48 ×Acc. 50 for mean PDI.*

Plate 1. *Maintenance of aphid (Aphis gossypii Glover) culture on cotton plants.*

values, the accessions were categorized into different categories. The mean PDI in F3 families in each disease reaction category derived from crosses viz., Acc. 1 x Acc. 50, Acc. 3 x Acc. 50 and Acc. 48 x Acc. 50 showed significant difference based on P value. The results showed that P value is less than F value indicating that there is significant difference between the categories *viz.*, immune, resistant, moderately resistant, moderately susceptible and susceptible reaction and disease scoring scale used for the screening of gherkin genotypes is efficient. There is substantial proportion of F3 families which are better than the resistant parent in all the three crosses viz., Acc.1 × Acc. 50, Acc.3 × Acc. 50 and Acc.48 × Acc. 50. The differential reaction of gherkin genotypes to CMV infection might be due to host biochemical's within the genotypes.

The analysis of all the F3 families for mean per cent disease index derived from all three crosses viz., Acc. 1 × Acc. 50, Acc. 3 × Acc. 50 and Acc. 48 × Acc. 50 (Fig. 1) showed there is substantial proportion of F3 families which are better than resistant parent Acc. 50.

Among 179 F3 progenies, seven of 179 F3 progenies were found Immune (17, 35 and 62 F_3 families derived from cross Acc.1-50, 26 and 34 F_3 family derived from cross Acc.3-50 and 11 and 47 F_3 family from Acc.48-50) (Plate 2a, 2b and 2c). However, 17 F3 progenies showed resistant reaction (R), 76 F3 progenies showed moderately resistant reaction (MR), 73 F3 progenies showed moderately susceptible reaction (MS) and 6 F3 progenies showed susceptible reaction (S) (Table 1). These F3 progenies were confirmed serologically for the presence of virus through DAS-ELISA. All the progenies showed positive reaction to CMV specific antisera except those F3 progenies which showed immune reaction to CMV. The grouping of gherkin F3 progenies on their reaction to the CMV is presented in Table 2. The estimate of mean PDI of F3 progenies classified into different response groups differed significantly suggesting the efficiency of scale and classification. The mean PDI in F_3 families classified into different response groups differed significantly justifying the classification of F3 progenies.

The immune and resistant F3 progenies identified were further assessed for response to CMV by aphid transmission (*Aphis gossypii* Glover) with one hour starvation followed by 10 min AAP and IAP. The similar reaction of accessions to CMV

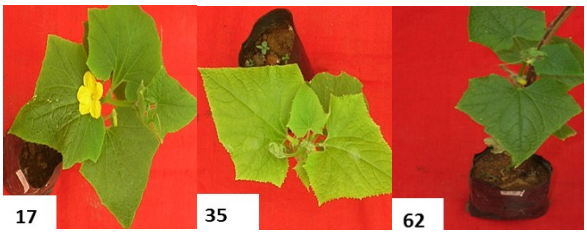

Plate 2a. *Gherkin F3 progenies derived from cross Acc. 1 x Acc. 50 showing immune reaction to CMV.*

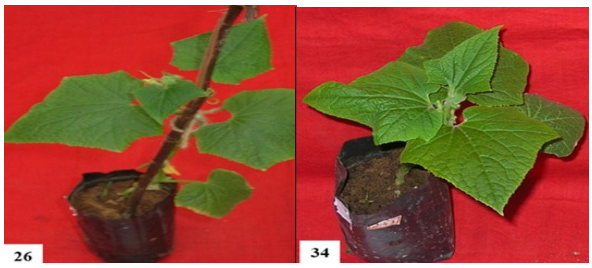

Plate 2b. *Gherkin F3 progenies derived from cross Acc. 3 x Acc. 50 showing immune reaction to CMV.*

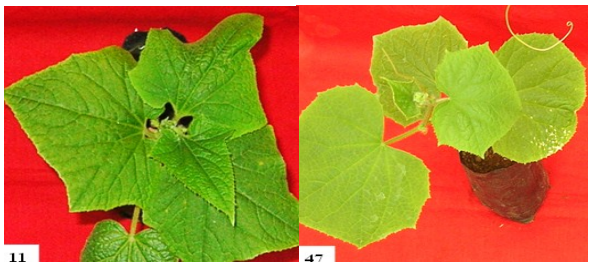

Plate 2c. *Gherkin F3 progenies derived from cross Acc. 48x Acc. 50 showing immune reaction to CMV.*

was observed as described in sap inoculation. The results of aphid transmission done on selected F3 progenies are presented in the table 3.

The disease reaction observed in gherkin genotypes is in accordance with the results obtained by Munshi *et al*. (2008). Among the 31 genotypes of *Cucumis anguria* var. *hardwickii* collected from Dehradun, Mussourie, Rishikesh and Kotwar in Uttaranchal, Mt. Abu in Rajasathan, Melghat, Khandla ghat, Raigadh Fort, Raigadh, and Ratnagiri in Maharashtra, Panhala and Jeypore in Orissa, Solan and Sirmur in Himachal Pradesh locations in India. The lowest mean *per cent* disease intensity (PDI) was recorded in IC-277048 (6.33%) while the highest PDI was observed in IC-331631 (75.33%) and all the four cultivated varieties (DC-1, DC-2, CHC-1 and CHC-2) showed very high PDI and susceptible disease reaction. Based on mean PDI, 8 genotypes were categorized as resistant, 13 as moderately resistant, nine as moderately susceptible and one as susceptible.

Among 43 genotypes screened for CMV resistance in gherkin (*Cucumis anguria* L.) , one genotype showed immune (I) reaction, 15 genotypes showed resistant (R) reaction, 15 genotypes showed moderately resistant (MR) reaction and 11 genotypes showed moderately susceptible (MS) reaction and one showed susceptible (S) reaction. The highest per cent disease incidence was observed in Acc. 48 (100%) and least was in Hyb. 11 (0.00%) genotype (Kavyashri, 2014). Ekbc *et al*. (2010) screened more than 350 melon accessions for CMV collected from different ecological parts of Turkey. Out of them, 67 melon accessions, sampled from this germplasm were tested for resistance; no resistant genotype was found to CMV. However, in Forty-five pepper breeding lines inoculated with CMV showed resistant in four lines as reported by Sun XiuDong *et al*. (2008).

The authors evaluated the F3 progenies to identify the source of resistance through mechanical inoculation and vector transmission. CMV sap was extracted from infected leaves showing characteristics CMV symptoms and confirmed through DAS -ELISA for its reactivity for CMV specific antisera. After confirmation with DAS-ELISA, the inoculum source of CMV was maintained on the gherkin plant and used the same plant as source of inoculum. The repeated experiments were conducted to define resistance. Similarly, vector transmission of CMV was done through aphid (*Aphis gossypii* Glover) with one hour starvation followed by 10 min AAP and IAP. The response of F3 progenies for level of resistance was found similar both in mechanical and vector transmission. This confirm the reaction of F3 progenies for CMV resistance, seven of 179 F3 progenies were found Immune (17, 35 and 62 F_3 families derived from cross Acc.1-50, 26 and 34 F_3 family derived from cross Acc.3-50 and 11 and 47 F_3 family from Acc.48-50), however, 17 F3 progenies showed resistant reaction (R).

Conclusion

Among 179 F3 progenies of gherkin (*Cucumis anguria* L.) screened for CMV in glasshouse condition, 7 F3 progenies were Immune, 17 Resistant, 76 Moderately Resistant, 73 Moderately Susceptible and 6 were Susceptible to CMV disease infection reaction. These Immune and Resistant F3 genotypes can be further used for the identification of molecular markers in breeding programme.

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