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SCREENING OF GHERKIN (*CUCUMIS ANGURIA* L.) GENOTYPES FOR CUCUMBER MOSAIC VIRUS RESISTANCE

V. V KAVYASHRI*,[1] N . NAGARAJU,[1] H. M RENUKA,[1] AND M. R. RADHA[2]
[1]Department of Plant Pathology,
[2] Department of Genetics and Plant Breeding UAS, GKVK, Bangalore-560 065
e-mail: kavyashrivv@gmail.com

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***Corresponding author**

ABSTRACT
Gherkin (*Cucumis anguria* L.) is an important cucurbitaceous vegetable crop cultivated in India in recent years. This crop is mainly affected by many viral diseases, among them cucumber mosaic virus (CMV) belongs to *Bromoviridae* is one of the most widespread and destructive disease on gherkin transmitted by several aphid species in non-persistent manner.To find out best source for resistance to CMV, forty three genotypes were screened under glasshouse condition mechanically through sap inoculation with a transmission varied from 0 to 100 %. One genotypes showed immune reaction (Hyb. 11), which also confirmed by serological and biological assay. The genotypes showed immune and resistant reaction would be utilized as donors to develop CMV resistant varieties

INTRODUCTION

Gherkin (*Cucumis anguria* L.) is an important cucurbitaceous vegetable crop grown in southern states of India *viz*., Andhra Pradesh, Karnataka and Tamilnadu for slicing and pickling. However, gherkins are being exported to other countries like Russia, followed by USA, Canada and Europe valued up to Rs.502 crores. Bottled gherkins pickled in vinegar contribute nearly 50% of the exports (Sukumaran, 2007). They are usually picked when 4 to 8 cm in length and pickled in jars or cans with vinegar or brine solution to resemble a pickled cucumber. The term can also be used to refer to the West Indian Burr Gherkin, a related species, originally from West Africa and introduced to the West Indies, probably by the Portuguese (Mugadur and Nittur, 2011). Viruses are the most common cause of diseases affecting cucurbits. These diseases resulted in losses through reduction in growth and yield due to distortion and mottling of fruit, making the product unmarketable. More than 25 viruses belonging to genera *Cucumo, Como, Tobamo, Poty* and *Ilarvirus* are known to infect cucurbits worldwide (Lovisolo, 1980). The mosaic disease in cucurbits was reported to cause by several viruses including members of genera *Cucumo, Como, Tobamo* and *Potyvirus* (Mukhopadhyay, 1985). The genus *Cucumovirus* is a major virus group infecting cucurbits, of which *Cucumber Mosaic virus* (CMV) is one of the most wide spread virus in the world infecting over 1000 plant species belonging to more than 85 families (Rossinck, 2002) causing yield losses as high as 40-60% (Varma and Giri, 1998). The virus is transmitted by *Aphis gossypii* and *Myzus persicae* in a non- persistent manner (Chandankar *et al*., 2013; Coudri *et al*., 1962). Since the virus has wide host range, spread rapidly by vector and lack of suitable host plant resistance, hence the management of CMV found difficult through cultural practices alone. There fore development of resistant varieties is widely perceived to be the only solution. However, utilization of genotypes as a resistance source against CMV in gherkin has not reported so far. Since the gherkins are being exported to overseas, it was thought to identify the resistant source as a best alternative Hence , germplasm accessions/hybrids of *C. anguria* were screened for CMV under glasshouse condition.

MATERIALS AND METHODS

Collection of gherkin genotypes/lines

The plant materials used in the present investigation comprised of 23 accessions and 15 hybrids of *C. anguria* which were collected from Department of Genetics and Plant Breeding, CoA, UAS, GKVK, Bangalore and maintained in Main research station, Hebbal, Bangalore.

Screening of Gherkin lines for resistance under glass house condition

The genotypes of *C. anguria* were screened for CMV resistance in an insect proof glasshouse. Ten seeds per accessions were sown in 6´×4´ polythene bag at the rate of single seeds per bag filled with pot mixture containing Soil + Sand + FYM. An equal number of susceptible cultivar Green long was sown in a similar manner as a control. The Gherkin lines were inoculated CMV mechanically through sap as described by Mandal *et al*. (2001). The Gherkin plants showing symptoms of mosaic pattern of dark green and yellow patches, blistering

and puckering of leaves, were collected from naturally infected plants from the fields and maintained on gherkin cv. Green long in glass house by sap inoculation as source of inoculum (Fig. 1). Young leaves of gherkin with typical symptoms of CMV were ground in a pestle and mortar with 0.05M phosphate buffer (pH 7.0) containing 0.2% sodium sulfite in the ratio of 1:5 (gm:ml) leaf buffer. The sap was then filtered through a double layered muslin cloth and collected in a beaker. About 1.0% of Celite was added to the sap as an abrasive to rupture the epidermal cell to gain entry of virus. This inoculum was applied with a cotton swab on the young cotyledon leaves of 12-15 day old test plants. After inoculation the pants were lightly misted with autoclaved distilled water and maintained in the insect proof cages for symptom expression.

Estimation of percent disease index (PDI)

The individual inoculated plants were monitored for scoring disease progress at 10 day intervals for a period of 30 days after inoculation (DAI). The symptoms were scored on a scale of 0-5 (Bos, 1982), where, 0 indicates no symptoms; 1 indicates very light mottling of older leaves and dark green colour in younger leaves; 2 indicates light and dark green areas associated with veins; 3 indicates mosaic, blistering and puckering of leaves; 4 indicates distortion of leaves and 5 indicates stunting of the plants with negligible or no flowering. The PDI was calculated for each genotype using the following equation used by Silbernagel and Jafari (1974) for measuring resistance to Beet curly top virus in snap bean (*Phaseolus vulgaris*) and modified by Bos (1982).

$$PDI = \frac{0n0 + 1n1 + 2n2 + 3n3 + 4n4 + 5n5}{nt(nC - 1)} X100$$

Where,

n0, n1, n2, .., n5 = No. of plants in score 0, 1, 2,.., 5, respectively,

nt = Total no. of plants,

nC = Total number of categories.

Based on the PDI values, the genotypes were classified into five categories *viz*., Immune (I) where PDI = 0; resistant (R) where PDI = 1-25%; moderately resistant (MR) where PDI = 26-50%; moderately susceptible (MS) where PDI = 51-75% and Susceptible (S) where PDI = 76-100% (Havey, 1996).

RESULTS AND DISCUSSION

Screening of gherkin genotypes for CMV resistance

The experiment was aimed to identify the source of resistance to CMV in the germplasm accessions and hybrids of *C. anguria*. The PDI of all genotypes were ranging from 0.0-100% (Table 1). One genotype (Hyb. 11) was categorized as immune, 15 as resistant, 15 as moderately resistant, 11 as moderately susceptible and one (Acc. 48) as susceptible (Table 2) based on PDI,Similar type of varietal evaluations was also well documented by Hobbs *et al*. (1996),Green house screening of pepper (*Capsicum annuum* L.) lines for reaction to cucumber mosaic cucumovirus (CMV) resulted the identification of a CMV-resistant line. The *C. annuum* line PBC 535 from the Asian Vegetable Research and Development Center, showed resistance to CMV isolates of different geographic locations. Similarly, the immune reaction of pepper line AF-97A and tolerance in AF-188, AF-1178, AF-98 A, AF-99A and AF-136A to CMV was observed by Frangioni *et al*. (2003).

Ambika *et al*. (2014) identified the resistance source for GBNV in tomato (*Solanum lycopersicon*). Tomato lines and wild species *Solanum perivianum* and *S. Lycopersicon* were screened under natural condition. Among them all the tomato

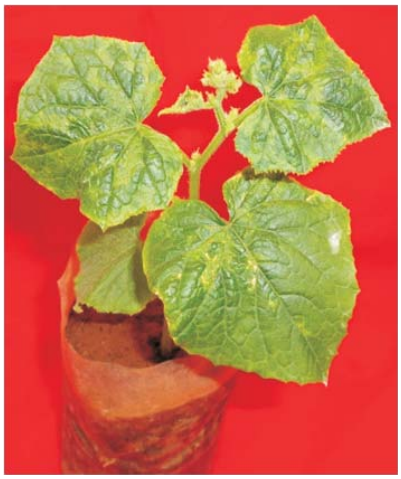

Figure 1: Gherkin plant showing mosaic symptom due to CMV infection

Figure 2: *Chenopodium amaranticolor* showing chlorotic local lesions due to CMV infection

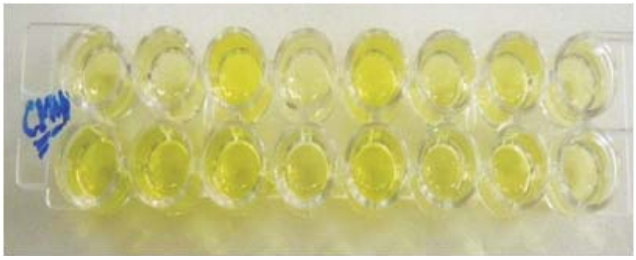

Figure 3 : Detection of CMV from a symptomatic gherkin plants through DAS-ELISA. Yellow colour - positive; Light yellow colour-week positive; No colour development - negative

Table 1 : Reaction of gherkin genotypes for cucumber mosaic virus*

Sl.No.	Genotypes/Cultivar	10DAI	20DAI	30DAI	MeanPDI (%)	Disease reaction
1.	Acc. 3	0.00	100.0	100.0	66.67	MS
2.	Acc. 22	0.00	20.00	40.00	20.00	R
3.	Acc. 23	20.00	40.00	60.00	40.00	MR
4.	Acc. 33	7.50	12.50	20.00	13.33	R
5.	Acc. 35	33.33	40.00	86.66	53.33	MS
6.	Acc. 39	50.00	75.00	75.00	66.67	MS
7.	Acc. 40	33.33	40.00	53.33	42.22	MR
8.	Acc. 41	20.00	30.00	45.00	31.66	MR
9.	Acc. 42	37.77	71.43	73.33	60.84	MS
10.	Acc. 43	0.00	34.00	22.00	18.66	R
11.	Acc. 45	12.00	16.00	20.00	16.00	R
12.	Acc. 46	0.00	10.00	20.00	10.00	R
13.	Acc. 47	6.67	13.33	13.33	11.11	R
14.	Acc. 48	100.0	100.0	100.0	100.0	S
15.	Acc. 50	12.00	20.00	36.00	22.66	R
16.	Acc. 51	16.00	20.00	40.00	25.33	R
17.	Acc. 52	13.33	33.33	86.67	44.44	MR
18.	Acc. 53	13.33	60.00	100.0	57.78	MS
19.	Acc. 54	0.00	95.00	95.00	63.33	MS
20.	Acc. 55	0.00	12.26	39.54	17.27	R
21.	Acc. 56	0.00	12.00	20.00	10.67	R
22.	Acc. 64	8.33	75.00	95.00	59.44	MS
23.	Acc. 66	3.57	91.67	96.25	63.83	MS
24.	Acc. 72	6.67	33.33	33.33	24.44	R
25.	Acc. 75	6.15	61.82	61.82	43.26	MR
26.	Acc. 77	8.50	53.33	80.00	47.27	MR
27.	Acc. 78	14.56	69.84	96.50	60.30	MS
28.	Acc. 80	9.42	83.65	97.75	63.61	MS
29.	Hyb. 1	16.66	33.33	50.00	33.33	MR
30.	Hyb. 2	12.00	24.00	35.00	23.67	R
31.	Hyb. 3	0.00	0.00	10.00	3.33	R
32.	Hyb. 4	0.00	10.00	20.00	10.00	R
33.	Hyb. 5	20.00	40.00	60.00	40.00	MR
34.	Hyb. 6	4.00	50.00	50.00	34.67	MR
35.	Hyb. 7	30.00	30.00	43.75	34.58	MR
36.	Hyb. 8	25.00	50.00	8.00	51.67	MS
37.	Hyb. 9	24.00	44.00	44.00	37.33	MR
38.	Hyb. 10	50.00	50.00	55.00	51.67	MS
39.	Hyb. 11	0.00	0.00	0.00	0.00	I
40.	Hyb. 12	6.67	53.33	73.33	44.44	MR
41.	Hyb. 13	0.00	20.00	50.00	23.33	R
42.	Hyb. 14	8.00	55.00	60.00	41.00	MR
43.	Hyb. 15	0.00	44.00	72.00	38.66	MR

*Experiment conducted under glass house condition by inoculating gherkin genotypes mechanically through infected CMV sap. DAI: Days After Inoculation, R: Resistant MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible

Table 2 : Grouping of gherkin genotypes/lines based on their reaction to cucumber mosaic virus*

PDI (%)**	Category	Genotypes/ Cultivars*
0	Immune(One)	Hyb. 11
1-25	Resistant(15)	Acc. 22, Acc. 33, Acc. 43, Acc. 45, Acc. 46, Acc. 47, Acc. 50, Acc. 51, Acc 55, Acc. 56, Acc. 72, Hyb. 2, Hyb. 3, Hyb. 4, Hyb. 13.
26-50	Moderately resistant(15)	Acc. 23, Acc. 39, Acc. 40, Acc. 41, Acc. 52, Acc. 75, Acc. 77, Hyb. 1, Hyb. 5, Hyb. 6, Hyb. 7, Hyb. 9, Hyb. 12, Hyb. 14, Hyb. 15
51-75	Moderately susceptible(11)	Hyb. 10, Hyb. 8, Acc. 80, Acc. 78, Acc. 66, Acc. 64, Acc. 54, Acc. 53, Acc. 42, Acc. 35, Acc. 3
76-100	Susceptible (one)	Acc. 48

*Experiment under glass house condition;** (Havey, 1996)

lines showed susceptible to GBNV, where as in wild species didn't showed any infection of GBNV. This was also confirmed by serological and biological assay.

Manjunatha *et al*. (2015) evaluated 100 cowpea genotypes against Bean common mosaic virus (BCMV) both under field and artificial condition and found three genotypes *viz*., IC-8966, V-5 and IC-202782 showed immune reaction.

Serological and biological confirmation of the virus in Gherkin genotypes

Forty-three genotypes including 28 accessions and 15 hybrids

were screened during *Rabi* 2013-14 under glasshouse condition. All the genotypes except Hyb. 11 showed characteristic visible symptoms of CMV disease. In order to confirm the presence of virus in the leaf sample, biological assay and DAS-ELISA was carried out, absence of virus in the sample was observed by expressing no chlorotic lesion on local lesion host *Chenopodium amaranticolor* L. (Fig. 2) and negative reaction in ELISA without colour development (Fig. 3). Herison *et al*. (2003) were evaluated 69 hot pepper (*Capsicum annuum*) lines for resistance to cucumber mosaic virus (CMV-02). Lines C1024, KA-2, PBC495 and C1042 showed consistently 0% disease intensity (DI), whereas lines *viz*., LV2323, Tit Paris and PBC068 recorded 10% DI. Forty melon cultivars collected from 17 Asian countries were screened for resistance to Indonesian isolate of cucumber mosaic virus (CMV-B2) by mechanical inoculation and confirmed serologically by ELISA. The resistance to CMV was found in five cultivars *viz*., Yamatouri, Miyamauri, Mawatauri, Sanuki-shirouri and Shinjong as reported by Daryono *et al*. (2003).

The immune and resistant category of gherkin genotypes screened under glasshouse condition for CMV could be utilized as donor parent for the development of resistant lines. The identification of markers linked to CMV resistance can also be better utalized for molecular screening of cucurbits as host -plant resistance. This approach may be the best method as eco-friendly perspective compared to pesticidal management.

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