



Evaluation of Different Cucumber Germplasm against Cucumber Mosaic Virus in Jammu, India

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Abstract: Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops of the family Cucurbitaceae, grown extensively in tropical and sub-tropical parts of the country. Cucumber production is under constant threat due to various fungal, bacterial and viral diseases. Among these, mosaic disease caused by cucumber mosaic virus (CMV) is the most predominant. Thus, the present study on screening of different cucumber germplasm against cucumber mosaic disease revealed that out of forty germplasm, two germplasm viz., Dasher II and Poinsett were resistant whereas CS-13, CS-16, CS-51, CS-54, Fumiko-10 and Don-1 were found moderately resistant during both the cropping season (2019 and 2020). The presence of CMV in infected plants were then confirmed serologically using DAS-ELISA. The resistant and moderately resistant cucumber lines from our study could be further used by farmers in cultivation under integrated production systems and by breeders in developing new varieties resistant to CMV.

Keywords: Cucumber Mosaic Virus, Screening, Detection, DAS- ELISA, Management

Cucumber production is adversely affected by many biotic and abiotic factors. Among various biotic factors, mosaic disease caused by cucumber mosaic virus (CMV) belonging to family *Bromoviridae* and genus *Cucumovirus*, is one of the most devastating and economically important disease of cucumber. Cucumber mosaic virus was first reported in 1916 by Doolittle and since then reported to cause disease in a variety of economically important agricultural, horticultural and ornamental crops under favorable environmental conditions. It has widest host range infecting over 1,200 species from 100 plant families. CMV is a positive sense tripartite virus having single stranded RNA, which is en-capsidated in a 28 nm icosahedral particle (Nault 1997). Cucumber plants may become infected with mosaic disease at any stage of growth, from emergence of the seedling to crop maturity and estimated to cause severe yield losses up to 40-60 per cent (Bananej and Vahdat 2008). CMV is transmitted by mechanical inoculation of plant sap and naturally transmitted by more than 80 species of aphids in non-persistent manner (Palukaitis and Garcia-Arenal 2003). *Myzus persicae* and *Aphis gossypii* are among the more efficient vectors for this virus (Tejashwani et al 2019). There are number of cultural control measures that can be used to prevent or reduce the spread of non-persistently transmitted viruses. Use of disease resistant crop varieties is regarded as an economical and durable method for controlling plant diseases, especially those caused by viruses. It is easy to adopt, cheap, and also environment

friendly. There are no adequate data regarding the evaluation of local germplasm for resistance against cucumber mosaic virus in Jammu region. Favorable environment for both vector and virus, lack of awareness about viral diseases among farmers and its wide host range results in severe epidemics of many of these plant viral diseases. Therefore, the present study was conducted to screen out different cucumber germplasm against CMV under field conditions.

MATERIAL AND METHODS

The study was carried out at an experimental farm of Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu (SKUAST- J) during the cropping season of 2019 and 2020.

Screening of cucumber germplasm: A total of forty cucumber germplasm collected from various sources (Table 1) were evaluated against cucumber mosaic virus in natural epiphytotic conditions in randomized block design with three replications. Recommended cultural practices were followed except for insecticidal sprays to encourage the vector population for the natural spread of the disease (Anonymous 2020). The data was recorded at 15 days interval on percent disease incidence starting from 30 days after transplanting and germplasm lines were divided into different categories (susceptible, moderately susceptible, moderately resistant and resistant) according to the disease rating scale by Shah et al 2011 (Table 2). The percent

disease incidence was recorded by using the following formula:

$$\text{Percent disease incidence (PDI)} = \frac{\text{No. of infected plants}}{\text{Total no. plants observed}} \times 100$$

Detection of virus through serological methods (DAS-ELISA): Leaf samples of cucumber plant showing characteristics symptoms of CMV were collected from the experimental field. These infected leaf samples were chopped into small pieces and grounded in pestle and mortar in phosphate buffer. Sap was filtered through double layered muslin cloth. Serological detection of these samples through DAS-ELISA (Double Antibody Sandwich- Enzyme Linked Immuno-sorbant Assay) described by Clark and Adams (1977) was then carried out under laboratory conditions at Division of Plant Pathology, SKUAST J.

RESULTS AND DISCUSSION

The forty germplasm lines were screened under natural epiphytotic conditions during the cropping seasons of 2019 and 2020 and were classified into four reaction groups based on the percent infected plants. The symptoms observed on CMV infected cucumber plants during the study were uneven yellow and green specks and patches on the leaves. Infected cucumber leaves also showed mottling and mosaic symptoms. In severely infected plants distortion of leaves was also observed. Out of forty germplasm, except for two, all other germplasm were susceptible to cucumber mosaic virus during both the cropping seasons of 2019 and 2020 (Table 3). Two germplasm viz., Dasher II and Poinsett were resistant whereas six germplasm viz., CS-13, CS-16, CS-51, CS-54, Fumiko-10 and Don-1 were moderately resistant. Twenty one germplasm were moderately susceptible while eleven germplasm were susceptible during 2019 and 2020. Many germplasm accessions showing resistance to CMV have been reported in cucumber by many other researchers. Munshi et al (2008) screened 31 accessions of *C. sativus* var. Hardwickii for CMV resistance and observed lowest mean percent disease intensity (PDI) in IC-277048 while the highest PDI in IC-331631. Akbar et al (2015) screened

seventeen germplasm in Pakistan and observed summer green, Local green, Khyber, Diamond, VEGAF1 and Yousuf as susceptible to mosaic disease of cucumber. Similarly, Shafiquique (2009) screened 12 varieties of cucumber against CMV in Faisalabad, Pakistan and found Beit alpha as moderately resistant with disease incidence of 16.26 per cent whereas Nandini-732 as highly susceptible.

All the germplasm screened during the cropping season of 2019 and 2020 were also tested serologically via DAS-ELISA for the confirmation of presence of cucumber mosaic virus (CMV). The advantage of this assay is that only virus particles are concentrated from infected plant extracts by the specific antibody coated in wells and other components are removed (Khan et al 2003). CMV specific antibody (Agdia, USA) was used to test the presence or absence of respective causal virus. Infected samples collected during screening were loaded into different wells of the ELISA plate coated with specific antibody. The data recorded on optimal density (OD value) i.e. absorbance value at 405 nm wavelength in both the year are presented in Table 4 and the overall results thus revealed that except for the samples from Dasher II and Poinsett all other screened lines were found infected with cucumber mosaic virus and showed positive reaction (presence of yellow color) with CMV specific antibody as the O.D values were more than twice the value of negative control reaction. Shetti et al (2012), Suresh et al (2013) and Hasan and Shams-bakhsh (2017) also detected cucumber mosaic virus (CMV) in cucumber (*Cucumis sativus*) and other cucurbits through direct plate and Dot-Enzyme Linked Immunosorbent Assay (ELISA).

Table 2. Disease rating scale for grading of varietal response to cucumber mosaic virus in cucumber germplasm

Disease incidence	Grade	Reaction group
0-10 %	R	Resistant
>10-30 %	MR	Moderately resistant
>30-50 %	MS	Moderately susceptible
>50 %	S	Susceptible

Table 1. Source of cucumber germplasm/lines

Source	Germplasm/lines	No. of entries
School of Biotechnology, SKUAST Jammu	CS-1, CS-13, CS-15, CS-16, CS-20, CS-22, CS-33, CS-34, CS-48, CS-51, CS-52, CS-54, CS-61, CS-67, CS-70, CS-73, CS-88, CS-103, CS-115, CS-149	20
Division of Vegetable Science, SKUAST Jammu	Cucumber Summer Green, Malini	02
Department of Agriculture, Talab Tillo, Jammu	Pusa Sanyog	01
Local Market Jammu	Fumiko-10, RK-180, R K 40, Poinsett, Khira Hybrid-1(KH-1), Khira 75, Dasher II, Nandini-732, Cucumber-DASH, Mahy Sylvia, Kirloskar, Prabhat, Don-1, Vardan, Garima Super, Cucumber Green Long, Local	17

Table 3. Screening of different germplasm of cucumber against cucumber mosaic disease under field conditions (2019 and 2020)

Germplasm	Per cent disease incidence						Mean (%)		Grade
	30 DAT		45 DAT		60 DAT		2019	2020	
	2019	2020	2019	2020	2019	2020			
CS-1	37.50	33.33	45.83	45.83	54.16	54.16	45.83	44.44	MS
CS-13	16.66	20.83	25.00	29.16	29.16	33.33	23.60	27.77	MR
CS-15	25.00	29.16	41.66	37.50	54.16	50.00	40.27	38.88	MS
CS-16	16.66	20.83	29.16	25.00	37.50	33.33	27.77	26.38	MR
CS-20	33.33	29.16	41.66	37.50	50.00	54.16	41.66	40.27	MS
CS-22	33.33	25.00	45.83	41.66	54.16	45.83	44.44	37.49	MS
CS-33	37.50	33.33	41.66	41.66	45.83	50.00	41.66	41.66	MS
CS-34	45.83	45.83	54.16	50.00	58.33	58.33	52.77	51.38	S
CS-48	29.16	20.83	37.50	33.33	41.66	37.50	36.10	30.55	MS
CS-51	20.83	16.66	29.16	25.00	33.33	37.50	27.77	26.38	MR
CS-52	25.00	29.16	33.33	33.33	37.50	37.50	31.94	33.33	MS
CS-54	25.00	12.50	25.00	20.83	29.16	25.00	26.38	19.44	MR
CS-61	29.16	25.00	41.66	37.50	50.00	45.83	39.58	36.11	MS
CS-67	45.83	37.50	58.33	54.16	58.33	62.50	54.16	51.38	S
CS-70	41.66	45.83	54.16	54.16	62.50	58.33	52.77	52.77	S
CS-73	20.83	29.16	33.33	29.16	37.50	33.33	30.55	30.55	MS
CS-88	25.00	37.50	41.66	50.00	54.16	54.16	40.27	47.22	MS
CS-103	29.16	33.33	45.83	45.83	50.00	54.16	41.66	44.44	MS
CS-115	16.66	25.00	33.33	33.33	45.83	41.66	31.94	33.33	MS
CS-149	37.50	25.00	45.83	37.50	54.16	50.00	45.83	37.50	MS
Cucumber summer green	41.66	41.66	50.00	54.16	62.50	62.50	51.38	52.77	S
Fumiko-10	20.83	16.66	25.00	25.00	25.00	29.16	23.61	23.60	MR
RK-180	37.50	37.50	41.66	50.00	50.00	54.16	43.05	47.22	MS
R K 40	41.66	45.83	54.16	50.00	62.50	58.33	52.77	51.39	S
Poinsett	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	R
Pusa Sanyog	25.00	29.16	33.33	33.33	37.50	37.50	31.94	33.33	MS
Khira Hybrid-1(KH-1)	25.00	25.00	41.66	37.50	45.83	41.66	37.49	34.72	MS
Khira 75	37.50	41.66	37.50	50.00	54.16	50.00	43.05	47.22	MS
Dasher II	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	R
Nandini-732	41.66	41.66	54.16	54.16	58.33	58.33	51.38	51.38	S
Cucumber-DASH	29.16	29.16	37.50	29.16	41.66	41.66	36.10	33.32	MS
Malini	45.83	45.83	54.16	58.33	66.66	62.50	55.55	55.55	S
Mahy Sylvia	37.50	37.50	45.83	50.00	50.00	54.16	44.44	47.22	MS
Kirloskar	29.16	25.00	41.66	33.33	54.16	50.00	41.66	36.11	MS
Prabhat	25.00	29.16	41.66	45.83	50.00	50.00	38.88	41.66	MS
Don-1	20.83	20.83	29.16	25.00	37.50	33.33	29.16	26.38	MR
Vardan	45.83	41.66	58.33	54.16	62.50	62.50	55.55	52.77	S
Garima Super	41.66	45.83	54.16	54.16	58.33	58.33	51.38	52.77	S
Cucumber Green Long	50.00	50.00	54.16	58.33	66.66	66.66	56.94	58.33	S
Local	50.00	54.16	62.50	66.66	70.83	70.83	61.11	63.88	S

Table 4. Serological detection of cucumber mosaic virus in different germplasm/lines (2019 and 2020)

Germplasm	No. of wells charged	OD value of CMV at 405 nm		Presence (+) or Absence (-) of virus
		2019	2020	
CS-1	2	0.1266- 0.1357	0.1830-0.2103	+
CS-13	2	0.1601-0.1797	0.2143-0.3970	+
CS-15	2	0.1422-0.1675	0.2503-0.2675	+
CS-16	2	0.2603-0.2887	0.1220-0.1387	+
CS-20	2	0.2160-0.2613	0.8830-1.1160	+
CS-22	2	0.1327-0.1520	0.4362-1.1270	+
CS-33	2	0.1877-0.2025	0.2650-0.2880	+
CS-34	2	0.1056-0.1215	0.1150-0.1376	+
CS-48	2	0.2033-0.2378	0.1560-0.1670	+
CS-51	2	0.2433-0.2680	0.2680-0.2980	+
CS-52	2	0.1520-0.1550	0.2440-0.2635	+
CS-54	2	0.1251-0.1775	0.1533-0.1775	+
CS-61	2	0.1620-0.1691	1.5310-1.691	+
CS-67	2	0.2903-0.3096	0.3565-0.3676	+
CS-70	2	0.2201-0.2894	0.1660-0.1894	+
CS-73	2	0.1590-0.1696	0.1866-0.2696	+
CS-88	2	0.1894-0.2011	0.1370-0.1894	+
CS-103	2	0.1686-0.2423	0.1330-0.1686	+
CS-115	2	0.2430-0.2968	0.1465-0.19021	+
CS-149	2	0.1591-0.1719	0.1560-0.16600	+
Cucumber Summer Green	2	0.1022-0.1436	0.2406-0.2641	+
Fumiko-10	2	0.9220-1.0270	1.0230-1.0274	+
RK-180	2	0.3210-0.3310	0.2630-1.033	+
R K 40	2	0.9622-1.0610	0.1822-1.061	+
Poinsett	2	0.0301-0.0462	0.0266-0.0288	-
Pusa Sanyog	2	0.1973-0.2050	0.2440-0.2907	+
Khira Hybrid-1(KH-1)	2	0.3801-0.3770	0.3662-1.0041	+
Khira 75	2	0.2160- 0.2811	0.2210- 0.2720	+
Dasher II	2	0.0182-0.0221	0.0161-0.0250	-
Nandini-732	2	0.2104-0.2690	0.1104-0.1420	+
Cucumber-DASH	2	0.2210- 0.2516	0.2310- 0.2396	+
Malini	2	0.1803-0.2350	0.2105-0.2450	+
Mahy Sylvia	2	0.2210- 0.2516	0.2370- 0.2530	+
Kirloskar	2	0.1860-0.2130	0.1800-0.2101	+
Prabhat	2	0.1706-0.1801	0.1702-0.1851	+
Don-1	2	0.1350-0.1720	0.1421-0.1520	+
Vardan	2	0.3213-0.3611	0.2213-0.3101	+
Garima Super	2	0.2658-0.2800	0.2654-0.2731	+
Cucumber Green Long	2	0.1866-0.1983	0.4066-0.7123	+
Local	2	0.2541-0.2960	0.5251-0.5901	+
Healthy tissue	2	0.0243-0.0409	0.0301-0.0470	-
Buffer	2	0.0227-0.0230	0.0220-0.0261	-

Table 5. Disease reaction of cucumber germplasm against cucumber mosaic disease under field conditions (2019 and 2020)

Reaction group	Percent disease incidence	No. of entries	Germplasm
Resistant	0-10 %	2	Dasher II, Poinsett
Moderately resistant	> 10-30 %	6	CS-13, CS-16, CS-51, CS-54, Fumiko-10, Don-1
Moderately susceptible	> 30-50 %	21	CS-1, CS-15, CS-20, CS-33, CS-22, CS-48, CS-52, CS-61, CS-73, CS-103, CS-115, CS-149, CS-88, RK-180, Pusa Sanyog, Khira Hybrid-1 (KH-1), Khira-75, Cucumber-Dash, Mahy Sylvia, Prabhat, Kirloskar
Susceptible	> 50 %	11	CS-67, CS-70, CS-34, Cucumber Green Long, Vardan, Garima Super, Malini, Nandini-732, Cucumber Summer Green, RK-40, Local

CONCLUSION

Out of forty germplasm/lines of cucumber screened against cucumber mosaic disease during 2019 and 2020 only two germplasm viz., Dasher II and Poinsett were found resistant whereas CS-13, CS-16, CS-51, CS-54, Fumiko-10 and Don-1 were moderately resistant. Thus, these resistant lines from our study can be further utilized in programs to explore resistant genes to develop CMV resistant cucumber cultivars and are suggested to be used as one of the management strategies to control the disease.

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