

# Varietal Reaction of Cucumber against *Cucumber mosaic virus*

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## Abstract

Family Cucurbitaceae is primarily found in the warmer regions of the world. It is the major family for economically important species, particularly edible fruits. In Pakistan cucurbits occupies an area of 28,600 ha with a very low production in Khyber Pukhtunkhwa due to many biotic and abiotic stresses. The reason is also the lack of growers' awareness about the diseases and the cultural practices adopted to provide favorable environment for development of epidemics. Viral diseases such as *Cucumber mosaic virus* (CMV) cause losses as high as 100%. Various control strategies are being used to control CMV. The aim of the current study was to screen out different varieties and to find the most resistant one against CMV. CMV isolate was collected from farmer's field at the site of TaruJaba during a survey of cucurbit crops. The identity of the virus was confirmed through DAS-ELISA using diagnostic kit (ADGEN, UK). Seventeen cucumber germplasm seeds were sown in earthen pots in which fourteen were germinated and exhibited characteristic symptoms of the virus while none of them showed resistance against CMV. Symptoms' expression was delayed in summer green and local green till 12 days post inoculation. While in khyber, Diamond, VEGAF1 and Yousaf, symptoms started to appear soon after inoculation categorizing them as highly susceptible. No resistance is found in available commercial germplasm, so more germplasm from different areas of Pakistan should be tested for resistance against CMV. If no resistance is found locally imported, germplasm can be evaluated for a source of resistance against the prevalent isolates of CMV.

## Keywords

CMV, DAS ELISA, Germplasm, Resistance

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## 1. Introduction

Cucurbitaceae or cucurbit family (also commonly referred to as the cucumber, gourd, melon, or pumpkin family) is primarily found in the warmer regions of the world. It is the major family for economically important species, particularly those edible fruits. It includes about 118 genera and 825 species [1]. They are well known for their nutritional value and are consumed in various forms, *i.e.*, salad (cucumber, gherkins, and long melon), sweet (ash gourd, and pointed gourd), pickles (gherkins), and deserts (melons). More than 90% content of cucumber is water. Besides its low caloric value, cucumber and other cucurbits are well served by potassium and folic acid but low in sodium. It contains vitamin A and C and also carotenoids when it is consumed with skin. The most economically important cucurbit crops worldwide [2] are watermelon (Mat sum) and cucumber (Anders).

Globally, cucurbits are grown on an area of 8.5 million hectares with production of 17.9 million tones [3]. China is leading in squashes and gourd production [4], while in India cucurbits share about 5.6% of the total vegetable production. In Pakistan cucurbit occupies an area of 28,600 ha with a total annual production of 261,306 tonnes [5], specifically in Khuber Pakhtukhwa where they occupies 1796 ha with estimated production of 17,256 tonnes [6]. The average yield of such crops (approximately 9 tones/ha) in the region is quite low. A number of biotic and abiotic diseases are important limiting factors that badly affect the yield of cucurbits.

The reason for such low yield of these crops is the lack of growers' awareness about the diseases and the cultural practices adopted to provide favourable environment for development of epidemics. The cultural practices adapted by the growers, lack of awareness of growers about viral diseases and their control, high population of weeds, and type of cropping pattern that includes the host crops of the prevalent viruses are some of the major contributing factors that result in a high incidence of viral diseases.

Cucurbits viral diseases are a problem worldwide that causes losses as high as 100% [7]. Almost 35 different viruses have been isolated from cucurbitaceous [8]. The most common viruses infecting cucurbits are from the CMV, ZYMV, WMV, PRSV, CGMMV and ZGMMV. These viruses occur in complex OR which may cause sole infection [9].

*Cucumber mosaic virus* (CMV) is one of the most important pathogen, which belongs to genus Cucumovirus in the family Bromoviridae. It is an economically important virus of agricultural crops and has the broadest host range known for any plant virus with approximately 1000 susceptible plant species [10], including monocots and dicots, herbaceous plants, shrubs, and trees. It has been found in all parts of the world, and numerous strains have been characterized. It has been very successful in rapidly adapting to new hosts and new environments.

Various control strategies are used for controlling these viruses and there is a dire need to evaluate different control strategies under each set of environmental condition and with different virus host combination to formulate an effective integrated control approach. Incidence of cucurbit viruses has been reported from Pakistan showing quite prevalence of these viruses in different cucurbit growing areas [11] [12]. There are no data regarding the evaluation of local germplasm for resistance against these viruses in Pakistan. 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. Use of resistance variety is the best and most economical method of viral disease control. It is easy to adopt and cheap, and also environment friendly. The present study was conducted to screen out available commercial cucumber germplasm against CMV.

## 2. Materials and Methods

### 2.1. Location of the Study

The study was conducted at the Department of Plant Pathology, The University of Agricultural Peshawar Pakistan, with the objective to screen different cucumber cultivars against (CMV).

### 2.2. Virus Isolate

CMV isolate was collected from farmer's field at TaruJaba during a survey of cucurbit crops. The identity of the virus was confirmed though DAS-ELISA using diagnostic kit (ADGEN, UK).

### 2.3. Germplasm Used for Screening

Seventeen germplasm of cucumber collected from local market and Agricultural Research Institute (ARI) Tarnab and were used for screening purpose. Twenty seeds of each cultivar were sown in earthen pots with two

seeds in each pot. Percent germination of each cultivar was observed after one week.

After germination, number of plants in each pot was reduced to one. One plant, out of ten, for each cultivar was used as control where inoculation was done using phosphate buffer only without crushing plant sample, while in the remaining 9 plants inoculation was done using virus infected sap.

## 2.4. Mechanical Inoculation

Mechanical inoculation was done on carborandum dusted cotyledonary leaves at cotyledonary leaf stage with the help of a cotton swab. The inoculum was prepared by crushing Ig of infected leaf in 10 ml phosphate buffer (2.4 gm of  $\text{KH}_2\text{PO}_4$  and 5.4 gm of  $\text{K}_2\text{HPO}_4$  in one liter water). Plants were washed with tap water after inoculation and kept in dark for overnight for symptoms development.

## 2.5. Symptoms Development and Indexing

After inoculation the plants were kept in screen house and were regularly monitored (twice a week) till four weeks post inoculation. The symptoms on the host plants were assessed using the following disease rating scale (1 - 5) as by [13].

## 2.6. Disease Rating Scale

1. = Highly resistant (no symptoms; 0% - 10% infection);
2. = Resistant (vein clearing after some time; 11% - 20%);
3. = Moderately resistant (vein clearing and mild mottle; 21% - 30%);
4. = Moderately susceptible (mild mosaic on few leaves; 31% - 40%);
5. = Susceptible (mosaic, wrinkling, mottling; >60%).

According to the disease rating scale the cucumber plants were divided into different categories.

## 2.7. Serological Assay

Finally all the samples were assayed by double antibody sandwich ELISA (DAS-ELISA) using commercial kits from ADGEN, UK to confirm the identity of the virus, as well as to check the possibility of any latent infection.

The following feature of the procedure, as recommended by the manufacturer kit, was used for the assay:

- 100  $\mu\text{l}$  of the antibodies were coated on the ELISA plates with the help of coating buffer and the plates were incubated at 37°C for 4 hours. Following incubation, the plates were washed three times with the help of washing buffer;
- Samples were homogenized in the extraction buffer and 100/l of the sap was applied to the wells. The plates were incubated at 4°C overnight followed by washing, thrice with the help of washing buffer;
- 100  $\mu\text{l}$  of conjugated antibodies were applied to the wells and plates were incubated at 37°C for 1 hr;
- The plates were incubated at about 25°C (or room, temperature) in dark;
- Data were recorded after 20, 30 and 60 minutes.

## 3. Results

Seventeen cucumber germplasm seeds were sown in earthen pots in which fourteen germinated and exhibited characteristics symptoms of the virus as described in **Table 1** and none of the tested germplasm showed resistance against CMV. Some of the germplasm are categorized as highly susceptible and some are moderately susceptible according to symptoms expression as described by [14].

At seven days of post inoculation, symptoms of CMV were observed in five germplasm that is in Khyber, Yousaf, VEGAF1, Waleed, and Diamond. Among the other germplasm symptoms started appearing after 12 days of post inoculation that was in (Betialpha, Punjab, Point pett-76, Baran, Summer green, Local green, Kurnar, Diamond 2 Fl, Sahill). No symptoms were observed on plants used as a control (inoculated with buffer only).

Symptoms expression was delayed in summer green and Local green till twelve days post inoculation while in Khyber, Diamond, VEGAF1, Yousaf, symptoms start appearing soon after inoculation, categorized them highly susceptible. Germplasm Beitalpha, Punjab, and Barandidnt show any symptom during the first two observations

**Table 1.** Reaction of cucumber germplasm after mechanical inoculation with CMV.

S. No.	Germplasm name	7 days	12 days	18 days	24 days	Disease rating	Symptoms appearance
1.	Khyber	3/9	6/9	8/9	9/9	5	Sm & M
2.	Beitalpha	0/9	0/9	8/9	9/9	4	Mm
3.	Punjab	0/9	0/9	8/9	9/9	4	Mm
4.	Point pett-76	0/9	3/9	6/9	9/9	4	Mm
5.	Baran	0/9	0/9	8/9	9/9	4	Mm
6.	Summer green	0/9	3/9	6/9	9/9	4	Mm
7.	Localgreen	0/9	3/9	6/9	9/9	4	Mm
8.	Kumar	0/9	3/9	6/9	9/9	4	Mm
9.	Yousaf	3/9	6/9	8/9	9/9	5	Sm & M
10.	VEGAF1	3/9	6/9	8/9	9/9	5	Sm & M
11.	Diamond 2F 1	0/9	3/9	6/9	9/9	4	Mm
12.	Sahill	0/9	3/9	6/9	9/9	4	Mm
13.	Iqbal	NG					NG
14.	Waleed	3/9	6/9	8/9	9/9	5	Sm & M
15.	Market more	NG					NG
16.	Holandgreen	NG					NG
17.	Diamond	6/9	8/9	9/9	9/9	5	Sm & M
18.	Control	0/9	0/9	0/9	0/9	1	NS

NG = No germination; NS = No symptoms; Sm & M = Severe mosaic & mottling; Mm = Mild mosaic.

and during the third observation 88%, (8/9), and almost all plants were infected during the 4th observation reaching to 100%. Among all of these germplasm, Diamond showed maximum susceptibility as the symptoms appeared soon after inoculation and all its 9 plants became infected. Thus showing high level of susceptibility compared to other susceptible cultivar.

#### 4. Discussion

*Cucumber mosaic virus* (CMV) is undoubtedly an important, serious and destructive disease in all growing countries of the world including Pakistan. In view of ubiquitous nature of CMV disease, 17 cucumber germplasm seeds were screened for resistance to CMV. These seeds were selected by the farmer's on locally based criteria over many years, which are strongly adapted for their local source conditions. The genotypes were classified into five reaction groups based upon % infected plants and ELISA test. These were: highly resistant, resistant, moderately resistant, moderately susceptible and susceptible. It is clear from the data that most of cucumber germplasm are moderately susceptible and if we observe [Table 2](#), the germplasm, Beitalpha, Punjab, Point pett-76, Baran, Summer green, Local green, Kurnar, Diamond 2 Fl and Sahill show mild mosaic and mottling, also the symptoms expression was delayed little bit hence they were considered as moderately susceptible the titer of the virus was also low in their leaves when checked through ELISA. The germplasm Khyber, Yousaf, VEGAF1 Waleed and Diamond, were found highly susceptible because symptoms start appearing soon after inoculation and they exhibited severe mosaic, wrinkling and mottling. The growth of these germplasm was also less as compared to others.

In Pakistan no systematic work has been done to determine the yield losses due to CMV diseases on crops. CMV is one of the major viral diseases recorded in world elsewhere including Pakistan [15] [16]. CMV persists all over the country and the main reason of its persistence is because of its inoculum which remains in the field

**Table 2.** The cucumber cultivars showing the degree of resistance/susceptibility against CMV.

S. No.	Scale/reaction	Cucumber
1.	Highly susceptible	Khyber, Yousaf, VEGAF1, Diamond Waleed
2.	Moderately susceptible	Punjab, Beitalpha, pointpett-76, Baran, Local green, Summer green, Diomond 2F I, Sahill
3.	Moderately resistant	—
4.	Resistant	—
5.	Highly resistant	—

for a long time and transportation of infested materials by human activities from one place to another, whereas for long area dispersal of the inocula is due to viruliferous aphids to a new cultivating area. Virus infected weeds within crop are serious threat because of the primary source of inoculum to the healthy ones [17]. Control can be achieved by elimination of the weeds by rouging and/or by using herbicides. However, viruses may persist in these weeds. Some viruses such CMV has been reported that spread through seeds of annual weed, *i.e.*, *Stellaria media* (Chickweed) [18].

Recent reports showed that aphid population has increased tremendously in the recent past due to favorable environmental conditions [19] and might have transmitted CMV to other Solanaceous hosts and has developed inocula for secondary infection. The insect vectors of CMV particularly aphids (*Aphis gossypii*) have broad host range [20]. As *Cucumber mosaic virus* is one of the major virus and is known to have broad host range and not easy to control it. Conventional measures like cross protection, eradication of infected plants, crop rotation, use of virus free plants and use of chemicals against vectors has been practiced since a long time to control plant viral diseases [21] [22]. The use of resistant varieties is considered as an economical and durable method for controlling viral diseases and therefore, management of viral diseases has always been focused on control of insect-vector by using chemicals.

## 5. Conclusion

It is concluded from the present findings that no resistance is found in available commercial germplasm, so more germplasm from different area of Pakistan should be tested for resistance against CMV. If no resistance is found locally imported, germplasm could be evaluated for source of resistance against the prevalent isolates of CMV. One main problem with germplasm evaluation is that some genotypes found resistance at one location turn out to be susceptible at another place [23]; therefore, resistant germplasms should also be studied for durable resistance in the future.

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