**Mixed infection of Cucumber mosaic virus and Capsicum chlorosis virus in pepper (*Capsicum annuum* L.)**


## Abstract

A survey was conducted on the basis of symptomatology for the screening of germplasm in the field of Department of Vegetable Science, Dr. YSPUHF, Nauni, Solanto identify the sources of resistance in pepper crop. The most prominent symptoms were mosaic, mottling, leaf narrowing, stunted growth, mosaic with chlorotic lesions, formation of rings with necrotic lesions and mosaic with mottling. DAS-ELISA was performed on symptomatic leaves and mixed infection of CMV and CaCV was found in UHF-Cap-22 and UHF-Cap-13. This study signifies presence of mixed infection with chlorotic lesions like symptoms and importance of DAS-ELISA in screening of pepper germplasm to determine the source of resistance to CMV and CaCV.


## Introduction

Peppers comprising of bell pepper, chillies and paprika are susceptible to a large number of viruses and causes yield losses upto 100 per cent (Kapoor 2012). The major viruses infecting pepper (*Capsicum annuum* L.) are Pepper veinal mottle virus (PVMV), Pepper mild mottle virus (PMMV), Potato virus Y (PVY), Cucumber mosaic virus (CMV) and Chilli vein mottle virus (CVMV) and among these viruses Cucumber mosaic virus (CMV) is the most prominent virus infecting peppers. Peppers have been reported to exhibit various symptoms which includes mottling, mosaic, vein clearing, stunted growth, reduced fruit size, chlorosis, ringspots, curling, necrotic spots and white streaks on green fruits (Kapoor et al. 2018, Gunes and Gumus, 2019). Considering the importance of CMV in pepper and severity of losses, a quick and reliable method for the detection of causal virus is of paramount importance.

## Material and Methods

### Collection of isolates

Pepper germplasm screening was conducted to identify the source of resistance to CMV disease insect-proof glasshouse conditions in the Department of Plant Pathology, Dr YS Parmar University, Nauni. Different varieties/ breeding lines available with the Department of Vegetable

Science were screened for their reaction to the virus isolate. These isolates were collected and brought to the laboratory for serological detection of causal viruses in samples by DAS-ELISA using CMV and CaCV antisera procured from BIOREBA, Switzerland.

**ELISA detection**

**Results and Discussion**

Samples were analyzed using an alkaline phosphatase-based direct DAS-ELISA following a modified protocol by Clark and Adams (1977). The assay utilized NUNC-immuno Maxisorp F96 microplates and CMV antisera from Bioreba, Switzerland. Anti-CMV antibodies were diluted 1:1000 in coating buffer with 200 µl added to each well followed by a 4-hour incubation at 30°C. After washing with PBS-Tween leaf extracts from test samples were added along with control and the plates were incubated overnight at 4-6°C. A 1:1000 dilution of ALP-conjugated antibody was then applied, with 5-hour incubation at 30°C. After additional washing, p-nitrophenyl phosphate (pNPP) substrate was added and color development was monitored for 30-90 minutes. The reaction was stopped with 3M NaOH, and absorbance was measured at 405 nm using a microplate reader (iMarkTM, BIO-RAD, USA).

**Symptomatology**

In this study, mixed infection of cucumber mosaic virus and capsicum chlorosis virus were found in peppers growing fields in the Department of Vegetable Science, Dr. YSPUHF, Nauni, Solan. The most prominent symptoms in pepper crop were mosaic, mottling, leaf narrowing, stunted growth, mosaic with chlorotic lesions, formation of rings with necrotic lesions, mosaic with mottling, chlorotic lesions.

**DAS-ELISA**

The pepper germplasm showing typical symptoms were screened by DAS-ELISA to confirm the presence of CMV and CaCV. On the basis of these symptoms, virus isolates were collected from the experimental farm, Department of Vegetable Science, Dr. YSPUHF, Nauni, Solan and loaded into the ELISA plate individually. After visual screening (Fig.1), the available germplasm

of peppers were screened serologically to ascertain the source(s) of resistance against *Cucumber mosaic virus*. Leaf samples from various pepper cultivars grown in an insect-proof glasshouse were collected for ELISA-based screening. The data presented in Table 1 revealed that out of forty eight varieties/breeding lines screened, nineteen tested positive, whereas thirty tested negative in DAS-ELISA for CMV, Sweet Banana variety recorded the least O.D. value of 0.228 followed by SB (0.252) and UHF-Cap-30 (0.274) and were found to be highly resistant, however, UHF-Cap-22 was found to be most susceptible variety with maximum O.D. value of 2.586 followed by UHF-Cap-13 with the O.D. value of 1.174 and California Wonder (UHF) with 1.054 O.D. value. Twelve lines showed no symptoms and also recorded negative serological reaction.

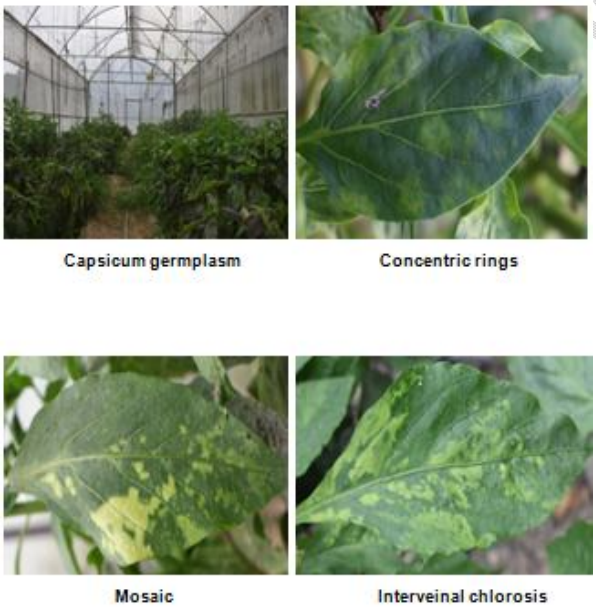


Fig 1.Symptoms observed during germplasm screening

Mixed infection of CMV and Capsicum chlorosis virus (CaCV) was recorded with six varieties/breeding lines during germplasm screening. These varieties/breeding lines were Solan Bharpur, UHF-Cap-20, UHF-Cap-22, UHF-Cap-13, PLDF (3) Med. and Cap-52 (Fig. 2 and 3). Need for ELISA based screening of pepper germplasm for identifying sources of resistance with objective of inclusion in future breeding program aimed at developing resistance against viruses in pepper have been a standard practice followed by a number of workers aiming at identifying

resistance sources in Capsicum spp. by a number of scientists (Grube et al.2000; Rashid et al.2007; Yao et al. 2013).

**Table 1: Screening of available germplasm of pepper against CMV and CaCV through DAS-ELISA**

| Sr. No. | Variety/Breeding line | Symptoms | O.D. value (405 nm) CMV | O.D. value (405 nm) CaCV |
|---|---|---|---|---|
| **1.** | California Wonder (UHF) | Mottle, mid vein distortion, mosaic | 1.054(+) | 0.252(-) |
| **2.** | Cap 4 | Mosaic and puckering | 0.849(+) | 0.201(-) |
| **3.** | Cap 27 | Mottling and mosaic | 0.721(+) | 0.278(-) |
| **4.** | Cap 52 | Mosaic and puckering | 1.018(+) | 0.550(-) |
| **5.** | IIHR-35 | Mottling | 0.983(+) | 0.272(-) |
| **6.** | KTC-182 | Vein banding, cupping and mottling | 0.916(+) | 0.142(-) |
| **7.** | UHF-Cap-20 | Mottling and leaf puckering | 0.729(+) | 0.244(-) |
| **8.** | UHF-Cap-21 | Mosaic and leaf deformation | 0.884(+) | 0.107(-) |
| **9.** | UHF-Cap-22 | Mosaic with mottling | 2.589(+) | 0.421(+) |
| **10.** | UHF-Cap-13 | Ring-spots with necrotic lesions | 1.174(+) | 0.532(+) |
| **11.** | UHF-Cap-20 | Mosaic with chlorotic lesions | 0.972(+) | 0.446(+) |
| **12.** | PLD F(3) Medium | Concentric chlorotic rings | 0.921(+) | 0.456(+) |
| **13.** | Cap4/1 | Mosaic with mottling | 0.819(+) | 0.343(+) |
| **14.** | Cap4/2 | Mottling | 0.992(+) | 0.201(-) |
| **15.** | Cap 2 HTP | Mosaic and leaf deformation | 0.988(+) | 0.225(-) |
| **16.** | F-29 | Puckering and leaf curling | 0.288(-) | 0.226(-) |

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|---|---|---|---|---|
| **16.** | Black | Yellowing and vein distortion | 0.333(-) | 0.246(-) |
| **17.** | ME | Mottling and leaf deformation | 0.464(+) | 0.275(-) |
| **18.** | F-28 | Blistering and yellowing | 0.397(-) | 0.256(-) |
| **19.** | T-11 | Mottling | 0.376(-) | 0.215(-) |
| **20.** | IIHR-39 | No symptoms | 0.377(-) | 0.225(-) |
| **21.** | SB×WE | Puckering and mottling | 0.458(+) | 0.226(-) |
| **22.** | CW | Mosaic | 0.478(+) | 0.245(-) |
| **23.** | YW | Mottling and cupping of leaves | 0.411(-) | 0.250(-) |
| **24.** | PLD (Yellow) | Mosaic and mottling | 0.335(-) | 0.213(-) |
| **25.** | PLDKS (M) | Mid vein distortion, mottling and stunting | 0.421(-) | 0.228(-) |
| **26.** | PLDKS (L) | Mid vein distortion and stunting | 0.353(-) | 0.210(-) |
| **27.** | SB | No symptoms | 0.252(-) | 0.205(-) |
| **28.** | IIHR-37 | Mottling | 0.312(-) | 0.240(-) |
| **29.** | IIHR-38 | Mottle and leaf deformation | 0.335(-) | 0.252(-) |
| **30.** | Cap4/3 | No symptoms | 0.325(-) | 0.260(-) |
| **31.** | Cap4/4 | Yellowing and leaf curling | 0.335(-) | 0.235(-) |
| **32.** | PLD (M) | No symptoms | 0.263(-) | 0.224(-) |
| **33.** | PLD (L) | No symptoms | 0.281(-) | 0.226(-) |
| **34.** | UHF (O-2) | No symptoms | 0.333(-) | 0.245(-) |
| **35.** | Solan Bharpoor | Mosaic, mottling and vein distortion | 0.489(+) | 0.250(-) |
| **36.** | Sweet Banana | Puckering and leaf curling | 0.228(-) | 0.213(-) |
| **37.** | KTC-181 | No symptoms | 0.359(-) | 0.228(-) |
| **38.** | Kandaghat Sel-9 | No symptoms | 0.292(-) | 0.210(-) |
| **39.** | Solan Selection-1 | No symptoms | 0.337(-) | 0.205(-) |

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|---|---|---|---|---|
| **40.** | KTC-12 | No symptoms | 0.329(-) | 0.240(-) |
| **41.** | CW× SB | No symptoms | 0.319(-) | 0.252(-) |
| **42.** | Solan Local | No symptoms | 0.321(-) | 0.260(-) |
| **43.** | UHF-Cap-23 | Mosaic and vein distortion | 0.324(-) | 0.235(-) |
| **44.** | UHF-Cap-24 | Puckering and leaf curling | 0.333(-) | 0.224(-) |
| **45.** | UHF-Cap-25 | Yellowing and leaf deformation | 0.360(-) | 0.215(-) |
| **46.** | UHF-Cap-26 | Mottling and puckering | 0.296(-) | 0.262(-) |
| **47.** | UHF-Cap-29 | Blistering and yellowing | 0.338(-) | 0.256(-) |
| **48.** | UHF-Cap-30 | Mottling | 0.274(-) | 0.270(-) |
| **49.** | Positive control | | **0.875** | **0.288** |
| **50.** | Negative control | | 0.212(-) | 0.150(-) |

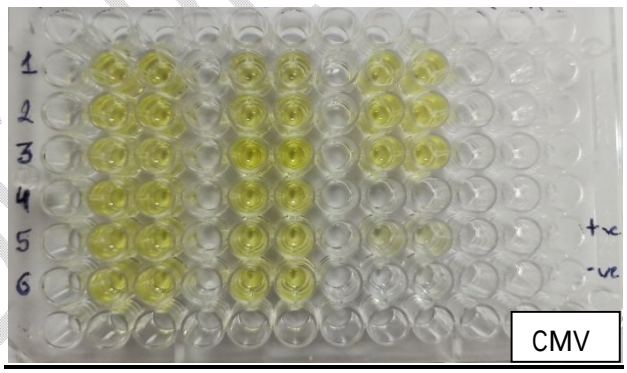


Fig2.Serological detection of CMV in pepper germplasm through DAS-ELISA

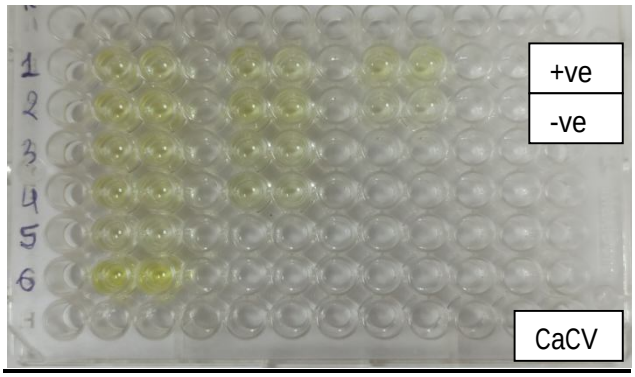


Fig3.Serological detection of CaCV in pepper germplasm through DAS-ELISA

Under Present investigations (Table1)Sweet Banana, UHF-Cap-30, F-29, Black, T-11, IIHR-39,YW,PLD(Yellow), PLDKS (M), PLDKS(L), SB, IIHR-37, IIHR-38, Cap4/3, Cap4/4, PLD(M), PLD(L), UHF(O-2), Kandaghat sel-9, Solan sel-1, KTC-12, KTC-181, California Wonder (UHF),Solan Local,UHF-Cap-23,UHF-Cap-24, UHF-Cap-25, UHF-Cap-26, UHF-Cap-29 and CW× SBvarieties/breeding lines were found to be free from infection of CMV and could be exploited for developing resistant cultivars.

**Conclusion**

Attempts were made to identify the source of resistance against Cucumber mosaic virus by screening available peppers germplasm using DAS-ELISA. A total of forty nine varieties/breeding lines were screened for the presence or absence of virus through DAS-ELISA. The study revealed that nineteen varieties/breeding lines tested positive and thirty tested negative for virus infection.These twenty seven varieties/breeding lines *viz.* Sweet Banana, UHF-Cap-30, F-29,Black,T-11,IIHR-39,YW, PLD(Yellow), PLDKS (M), PLDKS(L), SB, IIHR-37, IIHR-38, Cap4/3, Cap4/4, PLD(M), PLD(L), UHF(O-2), Kandaghat sel-9, Solan sel-1, KTC-12, KTC-181, California Wonder (UHF), Solan Local,UHF-Cap-23,UHF-Cap-24, UHF-Cap-25, UHF-Cap-26, UHF-Cap-29 and CW× SB could be used for developing resistance cultivars against CMV and CaCV in future.

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