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Serological Detection of Cucumber Mosaic Virus and Capsicum Chlorosis Virus in Pepper (*Capsicum annuum* L.) Germplasm

Naveen Katoch [a], Rishav Kumar [a*], Anil Handa [a], Chidembra Bhardwaj [a], Aditi Sharma [b] and Devinder Kumar Mehta [c]

[a] Plant Virology Lab, Department of Plant Pathology, College of Horticulture, Dr. YSP University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) –173230, India.
[b] Department of Silviculture and Agroforestry, College of Forestry, Dr. YSP University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) –173230, India.
[c] Department of Vegetable Science, College of Horticulture, Dr. YSP University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) –173230, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A survey was conducted on the basis of symptomatology for the screening of germplasm under insect proof glasshouse conditions in the Department of Vegetable Science, Dr. YSPUHF, Nauni, Solan to identify the sources of resistance in pepper crop. The most prominent symptoms were

**Corresponding author: E-mail: thakurrishav1193@gmail.com;*

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mosaic, mottling, leaf narrowing, stunted growth, mosaic with chlorotic lesions, formation of rings with necrotic lesions and mosaic with mottling. Forty-eight varieties or breeding lines were screened for the presence or absence of virus through DAS-ELISA. The study revealed that nineteen varieties or breeding lines tested positive and twenty-nine tested negative for virus infection. Sweet Banana variety recorded the least O.D. value followed by SB and UHF-Cap-30 whereas, UHF-Cap-22 was most susceptible variety with maximum O.D. value. Mixed infection of CMV and capsicum chlorosis orthotospovirus (CaCV) was recorded with six varieties or breeding lines during germplasm screening. This study signifies the presence of mixed infection with chlorotic lesions like symptoms and importance of DAS-ELISA in screening of pepper germplasm against CMV and CaCV.

Keywords: Serological detection; DAS-ELISA; *bell pepper; chlorosis virus.*

1. INTRODUCTION

Peppers (*Capsicum annuum* L.) commonly known as chilli, bell pepper or paprika belongs to Solanaceae family having more than 90 genera and 2500 species of flowering plants. Its origin is traced back to Tropical and Subtropical America. Among approximately 35 species in this genus, five species have been domesticated namely *C. annuum, C. baccatum, C. chinense, C. frutescens,* and *C. pubescens* [1]. Of all these worldwide, *C. annuum* holds the title of most extensively cultivated species. However, currently prevalent cultivated species are *C. frutescens* and *C. chinense* [2]. In India, Himachal Pradesh holds fifth position in terms of both its production and cultivated area and contributes approximately 2,850 ha of cultivated land with total annual production of 48.86 thousand metric tonnes [3]. Peppers comprising of bell pepper, chilies and paprika are susceptible to a large number of viruses and causes yield losses up to 100 per cent [4]. 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). 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 [4,5].

2. MATERIALS AND METHODS

2.1 Collection of Isolates

Pepper germplasm screening was conducted to identify the source of resistance to CMV and CaCV under insect-proof glasshouse conditions in the Department of Plant Pathology, Dr YS Parmar University, Nauni. Different varieties or breeding lines available with the Department of Vegetable Science, Dr. YSPUHF, Nauni, Solan 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 and G Biosciences, USA, respectively. CMV is transmitted by aphids from infected plant to healthy plants as they feed on the plant sap. The aphids transmit the virus in a non-persistent, stylet-borne manner hence these vector were being used as source of inoculum to infect the pepper plants.

2.2 ELISA Detection

2.2.1 DAS-ELISA

Pepper samples collected were subjected to Alkaline phosphatase based direct double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) following protocol of Clark and Adams [6]. The assay was performed using NUNC Maxisorp F96 polystyrene microplates and antisera against CMV supplied by Bioreba, Switzerland and CaCV by G Biosciences, USA. 200µL of the diluted CMV and CaCV antibodies in 1:1000 in coating buffer (10µL in 10ml) were added to each well. The plates were covered with foil and incubated at 30°C for 4 hours in a humid box. After removal of the coating antibodies, wells were washed thrice with PBS-Tween. Test samples, along with positive and negative controls, were added in 200µL aliquots and incubated overnight at 4-6°C. Following another washing step, alkaline phosphatase (ALP) conjugated antibodies (1:1000) were added to each well. After 5 hours of incubation at 30°C, wells were washed again. Freshly prepared p-nitrophenyl phosphate (pNPP) substrate was added (200µL per well) and plates were incubated in dark and humid box at room

temperature. The reaction was stopped with 50µL of 3M NaOH per well once a yellow color appeared (typically between 30-90 minutes). Results were assessed either by measuring absorbance at 405 nm using a microplate reader.

3. RESULTS AND DISCUSSION

3.1 Symptomatology

In this study, mixed infection of cucumber mosaic virus and capsicum chlorosis virus were found in peppers grown under insect proof glasshouse 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.

3.2 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 were tested positive, whereas, twenty-nine were 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.

Table 2 demonstrates the mixed infection of CMV and CaCV 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 (Figs. 2 and 3).

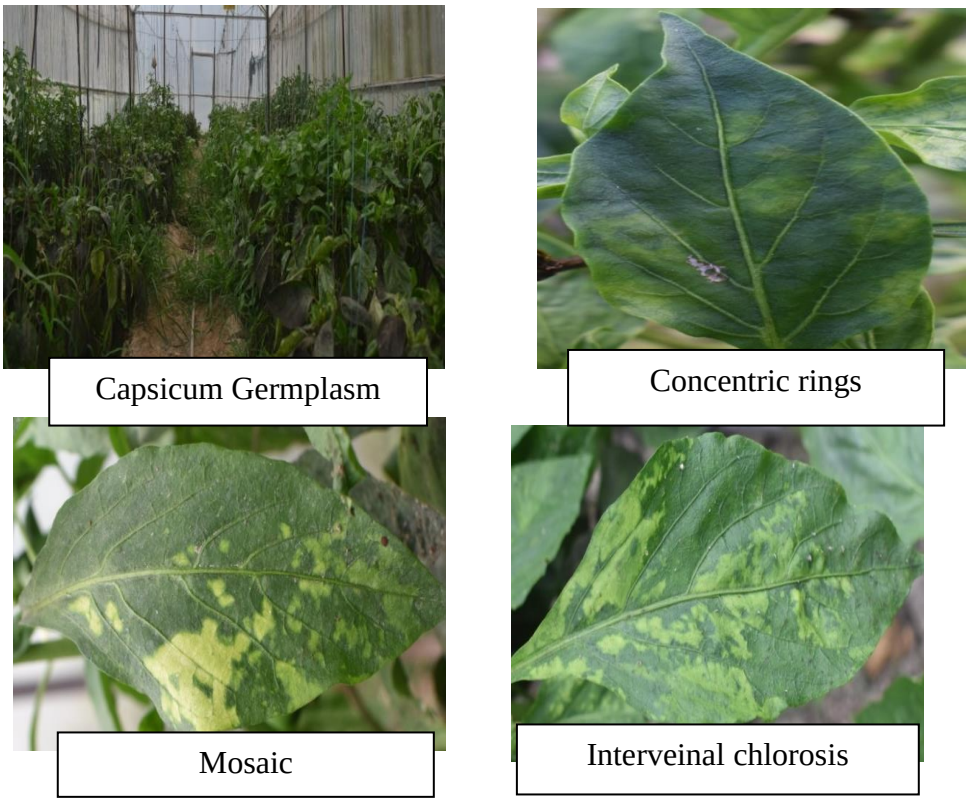

Fig. 1. Symptoms observed during germplasm screening

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(-)
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 Bharpur	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(-)
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(-)

Sr. No.	Variety/Breeding line	Symptoms	O.D. value (405 nm)CMV	O.D. value (405 nm)CaCV
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(-)

Table 2. Combined infection of cucumber mosaic virus and capsicum chlorosis virus

Sr. No.	Variety/Breeding line	O.D. Values At A405 against CMV antisera	O.D. Values At A405 against CaCV antisera
1.	Solan Bharpur	0.789(+)	0.664(+)
2.	UHF-Cap-20	0.729(+)	0.448(+)
3.	UHF-Cap-22	2.589(+)	0.396(+)
4.	UHF-Cap-13	1.174(+)	0.482(+)
5.	PLDF(3) Med.	0.921(+)	0.488(+)
6.	Cap-52	1.018(+)	0.587(+)
	Positive Control	0.875(+)	0.384(+)
	Negative Control	0.212(-)	0.161(-)

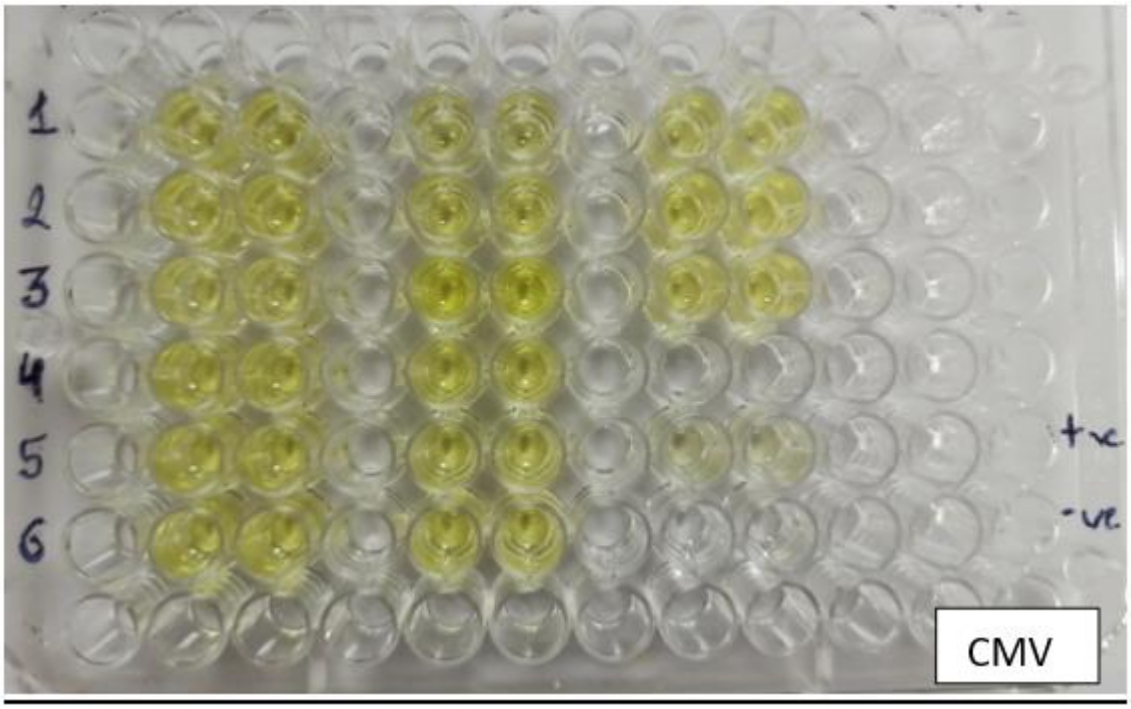

Fig. 2. Serological detection of CMV in pepper germplasm through DAS-ELISA

Under Present investigations (Table 1), 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 varieties/breeding lines varieties/breeding lines were found to be free from infection of CMV could be exploited for developing resistant cultivars.

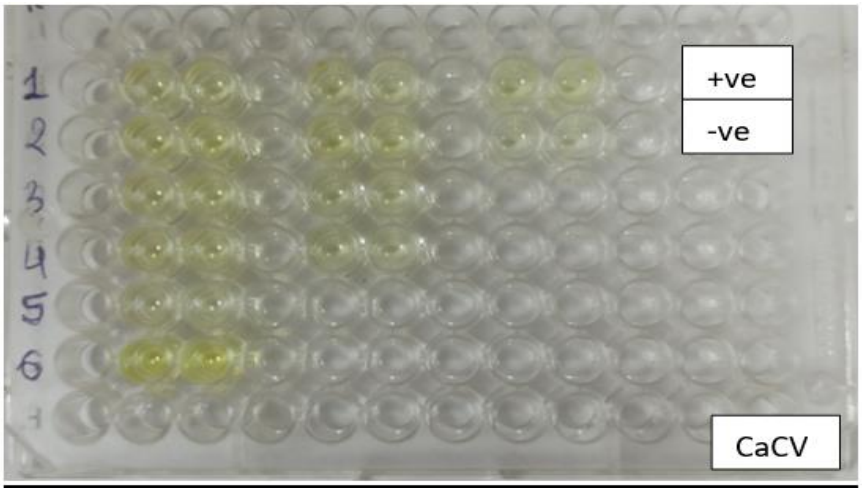

Fig. 3. Serological detection of CaCV in pepper germplasm through DAS-ELISA

4. DISCUSSION

The 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 aimed at identifying resistance sources in Capsicum spp. by a number of scientists [7-9]. These results are in accordance with other scientists with the following findings. Naresh et al. [10] screened fifty Capsicum genotypes for CMV resistance by mechanical inoculation. Among these eighteen immune, eight highly resistant, five resistant and two moderately resistant genotypes were identified against CMV. Fifty chilli genotypes were screened for resistance against ChiVMV through mechanical inoculation in an insect-proof glass house [11]. Six genotypes specifically BKS-03, BKS-06, BKS-14, BKS-28, BKS-33, and BKS-38 displayed a moderately resistant (MR) reaction. The remaining genotypes were found susceptible to the virus. Vinodhini et al. [12] recorded co-infection of CMV and CaCV in 5 samples. In these studies, mosaic mottling along with concentric chlorotic ringspot were suspected for mixed infection. The studies emphasize the 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.

5. CONCLUSION

Attempts were made to identify the source of resistance against cucumber mosaic virus by screening available peppers germplasm using DAS-ELISA. Forty-eight 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 twenty-nine tested negative for virus infection. These twenty-nine 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 resistant cultivars against CMV and CaCV in future.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Carrizo Garci C, Barfuss MHJ, Sehr EM, Barbozo GE, Samuel R and Mascone EA. Phylogenetic relationships, diversification and expansion of chili peppers (Capsicum, Solanaceae). Annals of Botany. 2016;118:35-51.

2. Saleh AA, Ragab MM, Ahmed EA, Abudabos AM and Ebeid TA. Effect of dietary zinc-methionine supplementation on growth performance, nutrient utilization, antioxidative properties and immune response in broiler chickens under high ambient temperature. Journal of Applied Animal Research. 2018;46:820-827.
3. National Horticulture Board. 2021. Area and Production of Horticultural Crops. Available:www.nhb.gov.in Accessed On:9th October 2022
4. Kapoor P. Detection of cucumber mosaic virus in bell pepper crop from Himachal Pradesh. Plant Disease Research. 2012;27:71-78.
5. Gunes N, Gumus M. Detection and characterization of Tomato spotted wilt virus and cucumber mosaic virus on pepper growing areas in Antalya. Journal of Agricultural Sciences. 2019;25:259-71.
6. Clark MF, Adams AN. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology. 1977;34:475-483.
7. Grube RC, Zhang Y, Murphy JF, Loaiza-Figueroa F, Lackney VK, Provvidenti R, Jahn MK. New source of resistance to cucumber mosaic virus in Capsicum frutescens. Plant Disease. 2000;84:885-91.
8. Rashid MH, Khalequzzaman KM, Alam MS, Uddin SA, Green SK.Screening of different sweet pepper lines against cucumber mosaic virus and chilli veinal mottle virus. International Journal of Sustainable Crop Production. 2007;2:1-4.
9. Yao M, Li N, Wang F, Ye Z. Genetic analysis and identification of QTLs for resistance to cucumber mosaic virus in chilli pepper (*Capsicum annuum* L.). Euphytica. 2013;193:135-45.
10. Naresh P, Reddy MK, Reddy PHC, Reddy KM.Screening chilli (*Capsicum spp*.) germplasm against cucumber mosaic virus, chilli veinal mottle virus and inheritance of resistance. European Journal of Plant Pathology.2016;146:451-64.
11. Chorgasti N, Naik RG, Renuka HM. Reaction of chilli genotypes against Chilli veinal mottle virus (ChiVMV) under glass house condition. International Journal of Current Microbiology and Applied Sciences. 2020;9:2401-2405.
12. Vinodhini J, Rajendran L, Abirami R, Karthikeyan G. Co-existence of chlorosis inducing strain of cucumber mosaic virus with tospoviruses on hot pepper (*Capsicum annuum*) in India. Scientific Reports. 2021;11:8796.

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