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ABBREVIATIONS

bp	Base- pairs (bp).
cDNA	Complementary deoxyribonucleic acid.
CI	Cytoplasmic inclusion.
CIA	Chloroform isoamyl alcohol
DBIA	Dot blot immunoassay.
D.E.P	Dilution end point of the infectivity of sap.
DNA	Deoxy ribonucleic acid.
EDTA	Ethylene diamintetraacetic acid.
ELISA	Enzyme- linked immunosorbent assay.
HSC	High- speed centrifugation.
L.I.V	Longevity of the infectivity of sap *in vitro*.
LSC	Low- speed centrifugation
MW	Molecular weight.
NBT	Nitroblue tetrazolium
nm	Nanometers.
NT	Nucleotide
NU	Nucleus.
PBS	Phosphate buffer saline.
PBST	P B S- Tween.
PCI	Phenol chloroform isoamyl alcohol
PCR	Polymerase chain reaction.
PEG	Polyethylene glycol.
PMMoV	*Pepper miled mottle virus.*
RNA	Ribonucleic acid.
RT	Reverse transcription.
SDS	Sodium dodecyl- sulphate.
SSC	Standard saline citrate
Taq	Thermus aquaticus
TBIA	Tissue blot immunoassay.
TBSV	Tomato bunshy spot virus
T.I.P	Thermal inactivation point.
V/V	Volume/ volume
W/V	Weight/ volume
WMV-2	Water melon mosaic virus

SUMMARY

Part I: Isolation and identification:

An isolate of PMMoV was obtained from naturally infected pepper plants grown in Kafer El-Sheikh Governorate, exhibiting different patterns of mottling, chlorosis with leaf distortion and stunting. The isolated virus was identified on the basis of host range, symptomatology, stability in crude extracts, modes of transmission, serological reaction, inclusion bodies, electron microscopy and molecular biology studies.

The results obtained could be summarized as follows:

1- Host range studies revealed that the isolated virus infected thirteen plant species and cultivars belonging to tow families.

The virus reacted systemically on all pepper cultivars tested (*C. annum* cv. california wonder, *C. frutescens*. cv. chilli) and *N. clevelandii*. However it becomes systemic in *Petunia hybrida* without causing any apparent symptoms. Moreover, the virus produced chlorotic local lesions on the inoculated leaves of *C. amaranticolor* and *C. quinoa*, and necrotic local lesions on *D. metel, D. stramonium, N. tabacum* cv. White Burley *N. sylvestris* and *N. glutinosa*. Whereas *N. tabacum* cv. Samsun and *Ocimum basilicum* were symptomless.

2- The virus was inactivated after heating for 10 min at 95°C but not at 90°C. The dilution end-point was between 10^{-7} and 10^{-8}. Virus infectivity *in vitro* was maintained more than 30 days storage at room temperature (26- 28°C).

3- The virus under study was transmitted mechanically and by infected seeds but cannot be transmitted by *M. persicae and Aphis babae.*

4- The identification of the virus was confirmed serologically by indirect ELISAA using TMV specific antiserum. Positive reaction obtained indicated that the virus under study was related serologically to tobamovirus.

5- Light microscope examinations revealed amorphous cytoplasmic inclusions (X- bodies) PMMoV infected cells.

Part II: Purification and antiserum production:

1- The virus was purified from infected plants using the purified procedure.

The UV-obsorption spectrum had a maximum at 260 nm and a minimum at 248 nm. The ratios of A_{max}/ A_{min} and A_{260}/ A_{280} were 1.11 and 1.21, respectively. The yield of purified PMMoV preparation was about 16.04 mg/100g of infected tissue.

2- Electron micrograph of purified virus preparation revealed rod-shaped particles of about 312 nm long and 18 nm width typical of tobamoviruses.

3- Polyclonal antibodies raised against PMMoV has a maximum titer of 1: 1024 from bleeding taken , two weeks after the last injection.

The dilution end point of PMMoV in infected tissue extracts was 1/640 as determined by indirect ELISA.

4- Detection of PMMoV in infected plants could be readily applied by DBIA and TBIA on nitrocellulose membranes using the produced antiserum.

Part III: Molecular biology studies of PMMoV:

- RT-PCR amplification of a DNA fragment of PMMoV coat protein region was approximately 500 bp when the primer annealing temperature was 50 or 60 °C.
- PCR using PMMoV specific primers gave a 470 bp amplified product corresponding to the coat protein gene of PMMoV.
- Southern and Dot blot hybridization were used to establish the authenticity and specificity to the RT-PCR amplified products of PMMoV.
- Clones of PMMoV- E_2 isolate was sequenced revealing 470 base fragments with deduced coat protein consisted of 157 amino acids.
- The nucleotide sequence showed 99%, 98% similarity with (PMMoV- Salto BR12 & PMMoV Ge 1) isolates belongs to Brazil and Japan, respectively).Where the coat protein of PMMoV-E2 was closely related to TMV –cp of Japan and Korea (99%and 97%), respectively.
- Moreover, it appeared far from Tropica soda apple mosaic virus–cp (TSAMV) of Florida (USA) Tobamoviruses isolate.