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# Isolation and Characterization of *Peach Rosette Mosaic Virus* (PRMV) in Egypt

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*Peach rosette mosaic virus* (PRMV) was detected from naturally infected commercial peach trees growing under field conditions at El – Dakkhlia Governorate, using DAS – ELISA. The detected virus is recorded for the first time in Egypt. Virus identification was performed using symptom expression, host range, graft transmission, serological tests i.e. DAS – ELISA, TBIA and DBIA, physical properties, electron microscope (EM) and reverse transcription Polymerase Chain Reaction (RT– PCR). Field survey was carried out in commercial peach orchards during 2001 – 2003 in four different locations using DAS – ELISA. The average percentages of infection in three seasons were 17.5% and 3.1% in El-Dakkhlia and El-Behira respectively. Naturally PRMV – infected trees develop chlorotic spots, rosetting, mosaic, chlorotic mottling, leaf deformation and shortening of the internodes (rosette appearance). The virus causes chlorotic local lesions on *Chenopodium quinoa* Wild, *Ch. amarnticolor* Cost&Reyn and N. tabcum L.cv.White Burley. It also causes local infection followed by systemic chlorotic leaf spot on Petunia hybrida, *Lycopersicon esculentum* cv. Castle Rock and *Vitis labrusca* L. Typical chlorotic spots was expressed on the woody indicator GF305 leaves 30 days after double chip-budding. Stability experiments of PRMV showed that the thermal inactivation point was 60- 65°C, the dilution end point was 10-3 and the longevity in vitro 15 – 20 days at room temperature. Using specific antiserum, PRMV was detected in the tissues of infected trees by DAS- ELISA, TBIA and DBIA. RT – PCR was used to amplify fragment of PRMV cDNA using specific primers designed to amplify ~200bp of the coat protein gene as a molecular procedure for diagnosis. Electron microscopy of purified preparation of PRMV showed presence of isometric particles 28 nm in diameter. Ultrathin section for electron microscopy examination of infected peach leaves shows virus particles in vacuoles. Tubules structure scattered in the cytoplasm or associated with plasmodesmata was showen. Extensive severe degeneration of chloroplast and mitochondria structure as well as development of cell wall protrusions was observed.

## INTRODUCTION

*Peach rosette mosaic virus* (PRMV) is one of the most economically important viruses affecting stone fruit and grapevine (Ramsdell and Myers, 1978). PRMV is a member of the *Nepovirus* genus (Cadman, 1963). The virus was first recognized as the causal of a disease of peaches (*Prunus persica* L.) in Michigan in 1917 (Klos *et al.*, 1976). PRMV is transmitted by Nematode [*Xiphinema americanum* and *Longidorus diadecturs*], mechanical inoculation, grafting and through seeds of grapevine cv.Concord (Ramsdell &Myers, 1978).PRMV appears as isometric particles non – enveloped 28nm with single stranded RNA positive sense of 8004 nucleotides. Occasionally, PRMV is occurred in Southwestern Ontario, Canada (Allen, *et al.*, 1982), in New York State (Ramsdell and Myers, 1974). It was observed only in Italy (Klos, *et al.*, 1976) as European country. PRMV was first reported in *Prunus persica*, *Vitis labrusca*; from North America by Cation (1933).Since then it has been reported to infect high bush Blueberry

*Vaccinium corymbosum* L. and many cultivars of Grapevine *Vitis labrusca* L. (Ramsdell and Myers, 1974 and Ramsdell and Gillett, 1981). PRMV is known to be able to infect most fruit trees species including Peach, Grapevine and Blueberries. However, the risk is high of rapid disease spread among Peach, Grapevine and Blueberries. Therefore, the purpose of this study is to isolate and identify a nepovirus (PRMV) infecting Peach.

## MATERIALS AND METHODS

### Source of virus isolate and orchard survey

Samples from peach trees showing symptoms suspected to be virus infection were collected from El-Dakahlia governorate (Metgamer) and North Sinai during spring and early summer season of 2001. The observed symptoms included chlorotic spots, rosette, mosaic, chlorotic mottling and distortion on early formed leaves and shortening of the internodes (rosette appearance). DAS – ELISA technique was conduct to test the collected samples using specific antiserum (supplied by SANOFI, Sante Animale, Paris, France) for stone fruit viruses i.e. *Plum pox virus* (PPV), *Tomato ring spot virus* (ToRSV), *Prune dwarf virus* (PDV), *Peach rosette mosaic virus* (PRMV), *Prunus necrotic ring spot virus* (PNRSV) and *Apple chlorotic leaf spot virus* (ACLSV). Samples reacted with PRMV positively were used as a source for virus identification.

Orchard survey for viral infection was carried out during 2001 – 2003 on peach orchards to determine PRMV occurrence using double antibody sandwich enzyme linked immuno-sorbant assay (DAS-ELISA) technique. The survey was conducted in commercial peach (*prunus persicae*) in four Governorates, i.e. Monofia (Khatatba), El Behira (El-Nobaria), North Sinai (Rafah) and Dakahlia (Metgamer).

### Isolation and identification of PRMV

Leaves of PRMV – naturally infected peach trees, which reacted positively with DAS – ELISA, were used for virus isolation and identification. About 5 gm of naturally infected leaf tissues were ground in 10 ml of buffer (0.01 M phosphate buffer, PH 7.4 contaning 2.0% nicotine).then inoculated onto carborandom dusted leaves of *Chenopodium quinoa* wild and *Lycopersicon esculentum* cv. Castle Rock. The inoculated seedlings were kept in the green house and were observed for symptoms development. Single local lesion technique (Kuhn 1964) was used for biological purification of the virus isolate. A single local lesion formed on *Ch. quinoa* was used to inoculate tomato (Castle Rock) leaves. Infected tomato plants which reacted positively only against PRMV were served as source of virus inoculum in the subsequent experiments.

### 1 – Mechanical transmission and Host range

Nine plants species and cultivars belonging to four families (*Chenopodiacea, Cucubitaceae Solanaceae and Vitaceae*) were mechanically inoculated with infectious sap expressed from Castle Rock tomato plants.

Ten seedlings of each host plants i.e. *Vitis labrusca* L. *Chenopodium amaranticolor* Coste and Reyn, *Chenopodium quinoa* Wild, *Cucumis sativus* L. cv. Beitalpha, *Nicotiana benthamiana* Domin, *N.tabacum* L.cv White Burley, *Datura stramonium*, *Petunia hybrida* and *Lycopersicon esculentum* cv. Castle Rock were inoculated and maintained in the

greenhouse for symptoms development. Additional ten seedlings were left as a control. Aback inoculation into the indicator host plant (*Ch. quinoa*) and / or DAS - ELISA technique was used either to check the specificity of infection or symptomless infection in the tested plants.

**2 – Graft Transmission**

Buds from PRMV-naturally infected peach trees cv. swelling were double chip-budding on five healthy peach cv.GF 305 seedlings. Buds from infected grapevine (*Vitis labrusca* L) were also double side grafted on grapevine. All inoculated plants were examined for external symptoms expression and confirmed by serological tests.

**3 – Physical properties**

Thermal inactivation point (TIP). dilution end point (DEP) and longevity in vitro (LIV) for PRMV were measured according to Noordam (1973).Thirty plants of *Chenopodium quinoa* was used as assay host for the virus isolate.

**4 – Serological Tests**

*4.1. Enzyme Linked Immuno – Sorbent Assay (DAS – ELISA) test:*

*Peach rosette mosaic virus* infection was tested using DAS – ELISA technique demonstrated by Clark and Adams (1977). ELISA kits for PRMV (Completely ready to use) were supplied by SANOFI, Sante Animale, Paris, France. The procedure was used as described in the kit instructions. The results were scored by recording the absorbance at 405nm at Tecan.Spectra ELISA reader. Samples with an absorbance of at least twice that of the healthy controls were considered positive for the presence of virus.

*4.2. Dot blot immuno – binding assay (DBIA).*

The technique of DBIA described by Knapp *et al.* (1995). Fifty plants were tested for PRMV – infection. The infected crude sap was blotted on nitrocellulose membrane (NC membrane) then examined serologically. Positive results indicated by the development of purple color on the blotted membrane and negative reaction developed no color.

*4.3. Tissue blot immuno – binding assay (TBIA)*

Fifty plants were tested for PRMV – infection using the technique of indirect immuno-blotting was used according to Knapp *et al.* (1995).

**5 – Virus Purification**

The virus was purified according to the method of Dias and Cation (1976).One hundred and fifty gram of freshly cut infected *Chenopodium quinoa* leaves [as indicator and propagative host] collected 3 weeks after inoculation were used. The procedure included clarification with chloroform and two cycles of differential (for low speed centrifugation Beckman J – 21C centrifuge using JA – 20 rotor and Beckman L8 – 80M Ultracentrifuge using 80 Ti rotor for high speed centrifugation) and sucrose density gradient centrifugation using Beckman SW 25.1.

The UV- absorption spectra of the supernatants containing virus particles were measured to evaluate the purity and concentration using an

extension coefficient $A^{0.1\%}$260nm, 1 cm (unfractionated virus) of 10.

*A – Electron microscope examination (EM)*

The carbon-coated grids were floated on drops of purified virus preparation for 15 min, stained with 2% aqueous uranyl acetate for 2 min. and air dried. Grids were then examined with SEO (Sumy Electron Optics) TEM-100 at the Electron Microscopy Unit, Military Veterinary Hospital., Cairo, Egypt.

*B – Ultrathin section of virus-infected tissues*

Small portions of leaf veinules 1X1 mm from both infected and healthy peach plants were cut with razor blade. Sections were then fixed in 3% cold 0.1M phosphate buffer glutaraldhyde, pH 7.3 and send to the Electron Microscopy Unit, Military Veterinary Hospital. For ultrathin section examination.

**6 – Molecular Detection**

*6.1. Extraction of nucleic acids from peach tissues:*

Total RNA was extracted from infected and non – infected peach tissues using CTAB based protocol that described by Gibbs and Mackenzie, (1997). Seventy-five mg infected peach cv. Swelling were harvested and ground in 600µl of CTAB buffer [2 % (w/v) CTAB (Cetyltrimethylammonum bromide), 1.4 M NaCl and 0.1 M TRIS – HCl pH 8.0] containing 0.5 % β – mercaptoethanol and mixed well, incubated at 55°C for 30 min. And 400 µl of chloroform isoamyl alcohol (24:1) was added and vortexed then centrifuged at 12.000 rpm for 10 min. After centrifugation the supernatant was carefully removed to a clean eppendorf tube. One tenth volume of isopropanol was added and mixed well. The mixture was incubated for 10 min at –20°C then centrifuged at 12.000 rpm for 10 min. The supernatant was removed carefully, and 1ml of 70% ethanol was added to the RNA pellet and centrifuged for 1 min. The supernatant was removed carefully and the pellet was air dried for 30 min. The RNA pellet was resuspended in 50 µl of sterile deionized $H_2O$ and stored at –20°C. The obtained RNA was used for RT – PCR.

**6.2. Reverse – transcriptase polymerase chain reaction (RT – PCR)**

Two primers were designed according to (Allan, *et al.*, 1999). 2.5µl of total nucleic acid was used as a template in reverse transcription reaction (RT) in order to synthesize complementary DNA (cDNA) using 1 µl of 10 µM [Complementary primer] PRMVC1 (5-CCATTGGCAACCGCC AGAGCTAC-3). 20 µl $H_2O$ at 70°C for 5min. The meloney murine leukemia virus reverse transcriptase (M-MLV) (Promega) was used in the cDNA synthesis. The RT reaction were performed in 40 µl containing 4 µl 5x RT buffer, 1µl 10mM dNTPs, 1µl RNAsin, 2.5 µl M-MLV – Reverse Transcriptase and 2 µl $H_2O$ at 42°C for 60min followed by incubation at 95°C for 3 min to inactivate the RNase and to denature the RNA-cDNA hybrids. The reaction was spun briefly and placed quickly on ice. PCR was performed in 50µl total volume containing mixture 1 [1 µl of 10 µM of (viral sense primer) PRMVV1 (5-GTGATGGAATATTTGGAC-3), 1 µl of 10 µM of (minus sense primer) PRMVC1 (5–CCATTGGCAACCGCCA

GAGCTAC-3), 0.5 µl 10 mM dNTPs, 10 µl $H_2O$] mixture 2 [2.5 µl 10x Expanded PCR buffer, 2.5 µl 25 mM $MgCl_2$, 0.5 µl expand enzyme mix 12 µl $H_2O$], mixture 1 was added to 2.0 µl cDNA followed by mixture 2. The PCR cycles were repeated 35 times under the following conditions: Denaturation at 94°C for 2 minutes, annealing at 60°C for 30sec. and extension at 72°C for two minutes. After the last cycle, elongation cycle was done at 72°C for 10 min. Amplified products were analyzed by 1.5% agarose gel electrophoresis in 0.5x TBE buffer (Tris-HCl 90 mM,Boric acid 90mM EDTA 2nM,pH 8) followed by staining with ethidium bromide for 10 min and de stained for 10 min in sterile deionized $H_2O$.

## RESULTS AND DISCUSSION

### Source of virus isolate and orchard survey

The virus under investigation was isolated from naturally infected peach trees cv. Swelling grown in El-Dakahlia (Metgamer) (Fig. 1) and North Sinai governorate (Rafah).Identification of the virus depended on symptomatology, mechanical transmission, host range, virus purification, morphology of virus particles and serological tests. All data obtained indicated that the virus under study was *Peach Rosette Mosaic virus* (PRMV).

Data presented in Table (1) indicate that the infection percentage in peach cultivars Florida Prince, Desert Red and Swelling in Monofia was 6.6%, 6.1% and 7.0% respectively and 4.5%, 3.1% and 0.0% respectively in El-Behira. The average of infection in peach cultivars Florida Prince, Desert Red and Early grand in North Sinai was 9.3%, 13.6%and 6.6% respectively, while in Dakahlia was 12.0%, 17.5% and 8.0% in peach cultivars Florida Prince, swelling and Metgamer respectively. The survey also indicate the dissemination of the virus was varied according to locations. Moreover, the percentages of infection were 6.5%in Monafia (Khatatba), 2.9%in El-Behira (El – Nobaria), 9.8%in North Sinai (Rafah) and 11.1%in El- Dakahlia (Metgamer) in the tested samples. These results agreed with (Ramsdell and Myers, 1978) they reported that PRMV is widespread transmitted through grafting and planting on old peach sites which formerly contained infected trees.

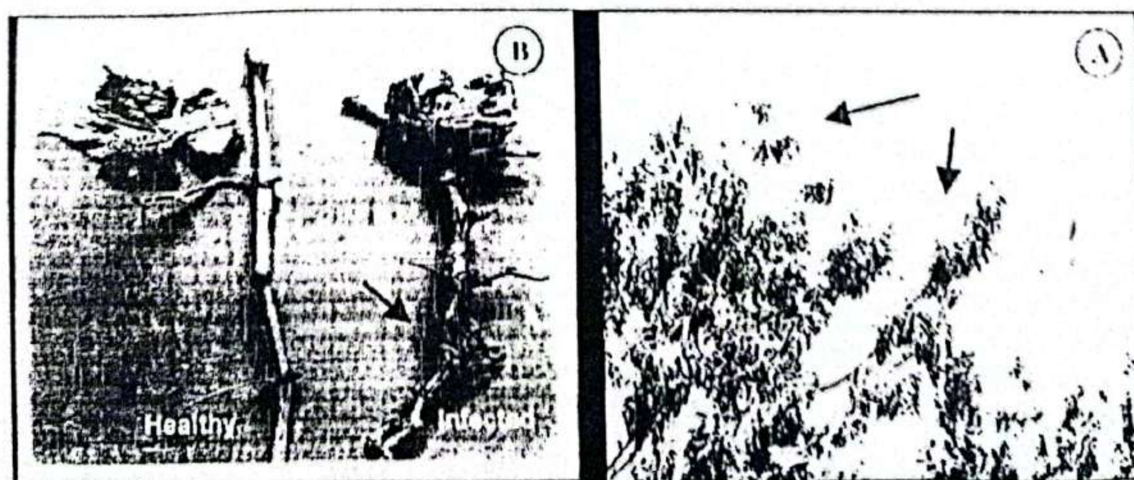



Fig. (1) :Naturally PRMV-infected trees
A. PRMV – infected peach tree cv. swelling showed chlorotic spots, rosette and mosaic.
B. PRMV – infected grapevine tree showed leaf deformation and short crooked cane internodes.

Table (1): Distribution of peach rosette mosaic virus (PRMV) infection in peach cultivars in different locations of Egypt

| Location | Monofia (Khatatba) | | | El-Behira EL-Nobria | | | North Sinai Rafah | | | Dakkahlia Metgamer | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar | *Tested | infected | % | *Tested | infected | % | *Tested | infected | % | *Tested | infected | % | *Tested | infected | % |
| FP | 330 | 22 | 6.6 | 600 | 27 | 4.5 | 354 | 33 | 9.3 | 390 | 47 | 12.0 | 1674 | 129 | 7.7 |
| DR | 420 | 26 | 6.1 | 480 | 15 | 3.1 | 300 | 41 | 13.6 | -- | -- | -- | 1200 | 82 | 6.8 |
| SW | 300 | 21 | 7.0 | 345 | -- | -- | -- | -- | -- | 324 | 57 | 17.5 | 969 | 78 | 8.0 |
| EG | -- | -- | -- | -- | -- | | 300 | 20 | 6.6 | -- | -- | -- | 300 | 20 | 6.6 |
| Metgamer | -- | -- | -- | -- | -- | -- | -- | -- | -- | 450 | 36 | 8.0 | 450 | 36 | 8.0 |
| Total | 1050 | 69 | 6.5 | 1425 | 42 | 2.9 | 954 | 94 | 9.8 | 1164 | 129 | 11.1 | 4593 | 345 | 7.5 |

- Data are based on enzyme linked immunosorbent assay of collections during 2001 - 2003. ELISA values absorbance at 405 nm
- (at Tecan.Spectra ELISA reader) ≥ were classified as positive reaction.
- * No. of tested plants in 3 years from 2001 to 2003.
- FP, Florida Prince; DR. Desert Red ; SW. Swelling ; EG. Early Grand.

**Identification of PRMV**

***1 – Mechanical transmission and Host range***

Nine plant species and cultivars belonging to four families were mechanically inoculated with the virus to determine the host range and differential host reactions. Data presented in Table (2) and (Fig. 2: A, B, C, D, E & F) illustrate the reaction of the plant species tested for PRMV infection. PRMV isolate produced chlorotic local lesion on the inoculated leaves of *Chenopodium amaranticolor* Coste and Reyn, *Chenopodium quinoa* Wild and *N.tabacum* L.cv White Burley. Systemic chlorotic local lesion on the inoculated leaves of *Petunia hybrida* and *Lycopersicon esculentum* cv. Castle Rock, leaf deformation on the inoculated *Vitis labrusca* L., were observed 12 – 14 days after inoculation. Results were confirmed by ELISA detection. The following spices and cultivars showed no symptoms as well as ELISA test failed to detect PRMV from them: *Cucumis sativus L.cv.* Beitalpha, *Nicotiana benthamiana* Domin and *Datura stramonium*. Such results confirmed the mechanical transmission mode of PRMV isolate and agree with the results of many investigators working on PRMV [Ramsdell and Myers, 1974; Dias, 1975; Dias and Cation, 1976 and Dias and Allen, 1980]. Egyptian isolate of PRMV could infect herbaceous hosts. Host range of Egyptian isolate appears similar to those reported for PRMV isolate by Ramsdell and Myers, (1974); Dias, (1975); Dias and Cation, (1976) and Dias and Allen, (1980). They reported that PRMV infects restricted species outside family Roseaceae and some of them were without symptoms.

***2 – Graft transmission***

PRMV-isolate was successfully double chip bud – transmitted from infected peach plants to indicator rootstock peach cv. GF 305. Typical symptoms were observed on grafted peach seedling cv. GF 305 after 4 weeks of inoculation. Also typical symptoms were appeared on grafted grapevine after 4 weeks of inoculation with infected grapevine (Fig. 2: G&H). No symptoms were observed on control plants grafted with healthy buds. Results were confirmed by DAS-ELISA. These results clearly indicated that the virus spread from field to field or region to region through scion buds. Such results in agreement with that obtained by Dias, (1975); Dias and Cation, (1976); Dias and Allen, (1980) and Ramsdell and Myers, (1974), they showed that PRMV could be transmitted through grafted trees.

***3 – Physical properties***

Data obtained showed that TIP of the virus ranged between 60 - 65°C, the virus lost its infectivity when the inoculum was diluted up to $10^{-3}$ and it could be active after storage 15 – 20 days at room temperature (25 – 28°C). These results agreed with Dias and Cation (1976) and Dias and Allen (1980) they recorded that the thermal inactivation point (10min) was 58 – 68 °C when the infectious sap was obtained from *Ch.quinoa*. The DEP, was slightly beyond $10^{-3}$ - $10^{-5}$.

They also found that the infectivity was retained at 20 – 25 °C for 15 – 25 days. This difference in the properties of our isolate may due to the strain characters and source of inoculums as well as bioassay hosts used. In this respect, Dias and Cation (1976), Dias and Allen (1980) concluded that the in *vitro* properties of PRMV were variable according to the strain and the species of plant in which the investigation is carried out

*4 – Serological Tests*

Fifty plants were tested using immuno-blotting assay and thirteen plants were reacted positively. Data presented in (fig. 3) Indicated that Immuno-blotting assay has the same sensitivity of ELISA test, it could be considered as fast and sensitive procedure that could be easily adapted to large- scale compared with other detection methods. Immuno – blotting assay was modified to make it simple, rapid and applicable for the detection of PRMV .The test was simple, rapid, and consistent throughout the growing period. It was also economical and convenient. The DBIA used only 1% of the antigen, 2 – 3% of antibody compared with ELISA. DBIA can also visualized without expensive automatic instruments and the nitrocellulose membranes can be stored for reference. Thus facilitating the introduction of certification tests in poorly equipped laboratories located close to production areas is very important. In addition, DBIA requires minimal training and can easily be preformed by individual farmers wishing to assess the quality of their own trees (Wang *et al.* 1998).

**5 – Virus Purification**

For Purification, only freshly harvested leaves were convenient; no purified suspensions are obtained from the frozen material. Virus yield was 1mg / ml / 150g of infected material. The yield of purified virus preparation was rather low comparing to that obtained by Dias and Cation, (1976) they reported that yields of purified virus were 1 to 3 mg /100g starting material. However, other results by Allan *et al.* (1999) found that purification of PRMV yielded 0.2 – 0.3 mg virus per 100 g of infected *Ch. quinoa*. The ultraviolet absorption spectrum of PRMV from several purified samples showed a curve typical for nucleoprotein (Fig. 4) with a maximum at 260 nm and a minimum at 243 nm. But no shoulder at 290 nm was observed. The value of $A_{260}/A_{280}$ was 1.7. These results agreed with Dias and Allen. 1980 and Allan. *et al*. 1999 they reported that $A_{260}/A_{280}$ ranged from 1.7 – 1.9 and 1.8 respectively.

**Table (2) :Reaction of some host range to infection by PRMV isolate**

| Family / Tested host | Reaction | ELISA Test |
|---|---|---|
| Chenopodiaceae | | |
| *Chenopodium quinoa* Wild | C.L.L. | + |
| *Ch. amarnticolor cost&Reyn* | C.L.L. | + |
| Cucurbitaceae | | |
| *Cucumis sativus* L.cv. Beitalpha | --- | - |
| Solanaceae | | |
| *Nicotiana benthamiana* Domin | --- | - |
| *N. tabcum* L.cv.White Burley | C.L.L. | + |
| *Datura stramonium* | --- | - |
| *Petunia hybrida* | S - C.L.s. | + |
| *Lycopersicon esculentum* cv. Castle Rock | S - C.L.s. | + |
| Vitaceae | | |
| *Vitis labrusca* L. | LD | + |

* C.L.L. Chlorotic local lesions * --- No symptoms * + Positive reaction * - Negative Reaction
* S. C.L.s Systemic chlorotic leaf spot * LD Leaf distortion

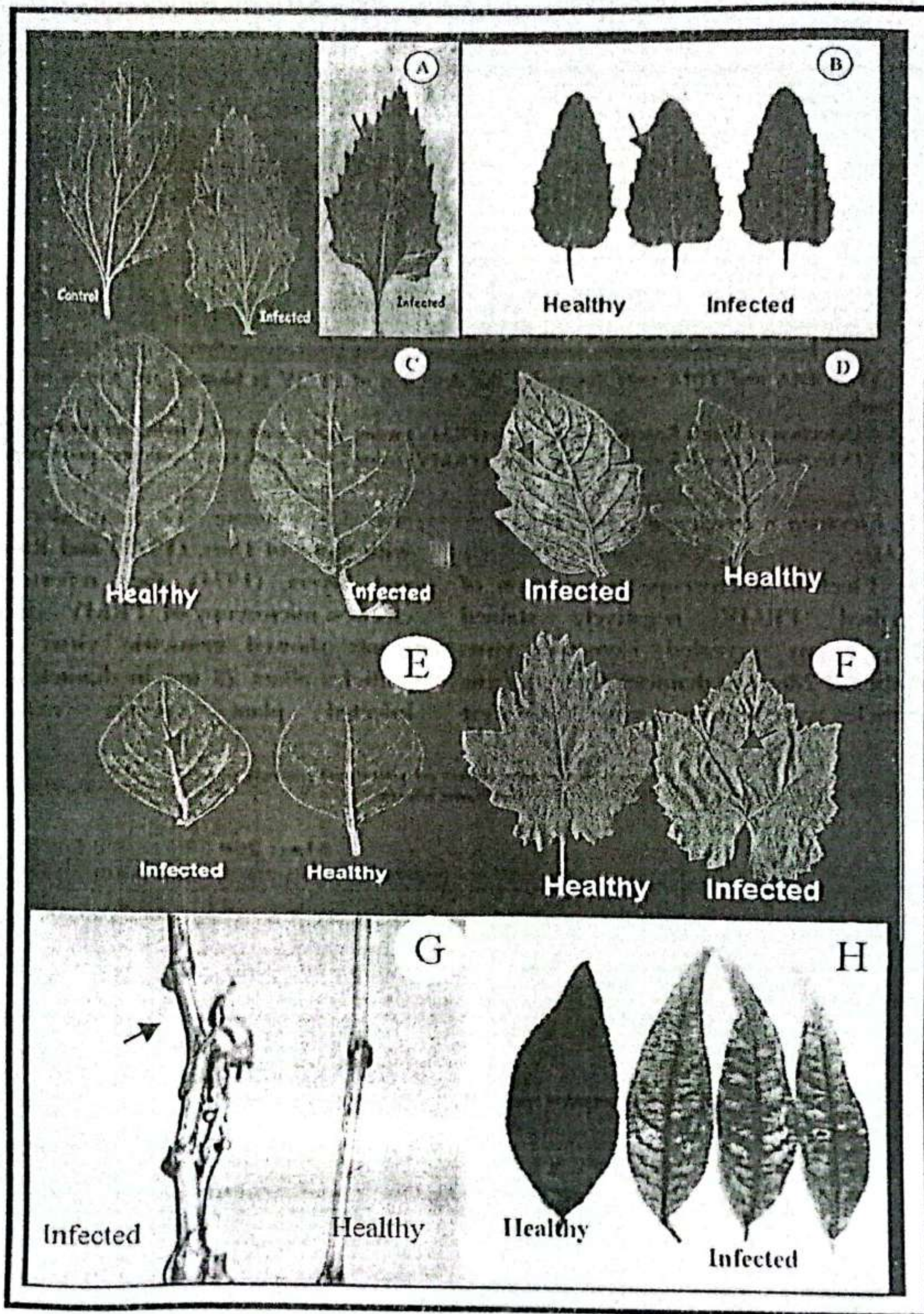



Fig. (2): Symptoms on some susceptible host to peach rosette mosaic virus.

A. *Chenopodium quinoa* Wild showing chlorotic local lesions.

B. *Chenopodium amaranticolor* Coste and Reyn showing chlorotic local lesions.

C. *N.tabacum* L.cv White Burley showing chlorotic local lesions.

D *Petunia hybrida* showing systemic chlorotic local lesions.

E. *Lycopersicon esculentum* cv. Castle Rock showing systemic chlorotic local lesions.

F. *Vitis labrusca* L. showing leaf deformation

G. *Vitis labrusca* L. showing shortens of the internodes (rosette appearance) caused by Peach Rosette Mosaic virus (PRMV).

H. Peach seedling cv. GF 305 showing chlorotic spots, mosaic, chlorotic mottling caused by Peach Rosette Mosaic virus (PRMV).

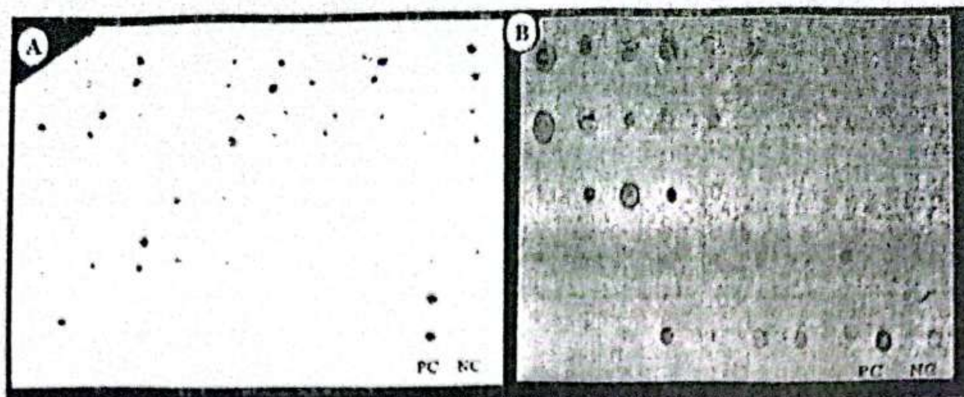



Fig. (3): DBIA and TBIA tests illustrating the detection of PRMV in blotted- cut leaves of different orchards.

A. Detection of Peach Rosette Mosaic virus (PRMV) using TBIA, pink color indicates positive reaction.
B. Detection of Peach Rosette Mosaic virus (PRMV) using DBIA, pink color indicates positive reaction.

### *A Electron microscope Examination (EM):*

Electron microscope examination of purified PRMV negatively stained preparations revealed isometric virus particles, 28nm in diameter (Fig. 5).Virus particles were measured using Video Test size 5.0 software. These results agreed with those of Dias, (1975) and Ramsdell and Myers, (1974), they reported that electron microscopy of PRMV – infected plants showed isometric virus – like particles about 28 nm in diameter in all infected plant species examined.

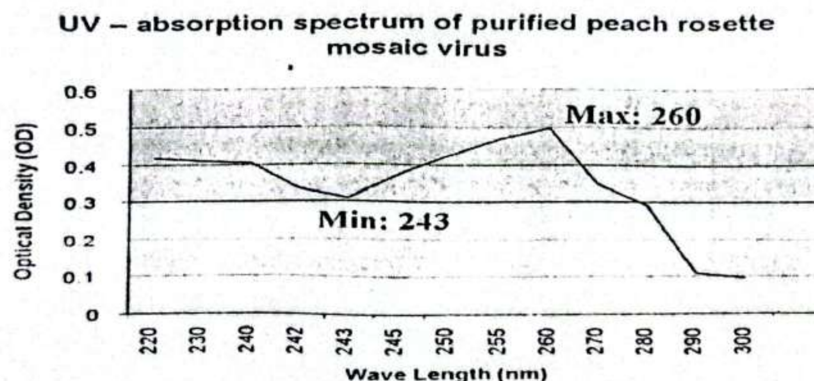



Fig. (4): UV – absorption spectrum of purified peach rosette mosaic virus.

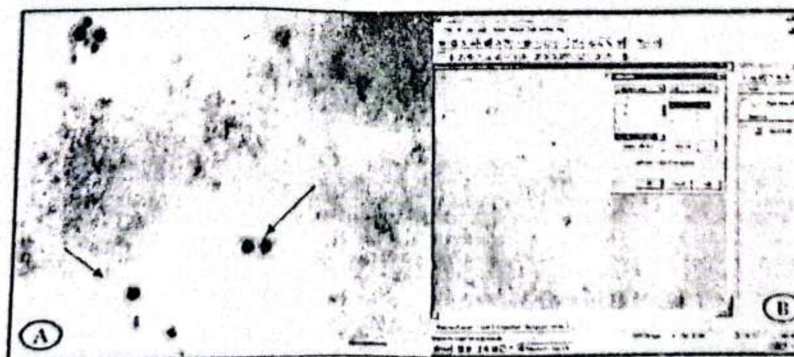



Fig. (5): A. Purified virus particle preparation stained with uranyl acetate and Lead nitrate. Bar represents 100 nm. (x 175 000). B. Measurement of virus particles using Video Test size 5.0 software.

***B – Ultrathin section of Virus infected tissues:***

Electron microscope examination of ultrathin section of peach leaves healthy and infected with PRMV was done. Healthy samples showed normal cell wall, nucleus, mitochondria and chloroplasts. The chloroplasts were ellipsoidal, elongated, with numerous thylakoids having a good granular organization, rare plastoglobuli, small starch little grains. (Fig. 6).However, infected samples showed that infected cells contain virus particles in cytoplasmic tubules, where the plasmodesmata and vacuoles had no morphological change in the cellular organization of meristemic cells. On the other hand extensive changes have been observed in infected mesophyll cells including severe degeneration of chloroplast structure and development of cell wall protrusions and the Mitochondria appeared to be round than normal (Fig. 6). These results confirmed with Gerola *et al.* (1966), Crowley *et al.* (1969), Robert *et al.* (1970) and Jones *et. al.* (1973).They reported that nepovirus – infected cells often contained virus particles in cytoplasmic tubules and no morphological changes have been reported in the cellular organization of meristematic cells. Including severe modifications of chloroplast `and Mitochondria structure.

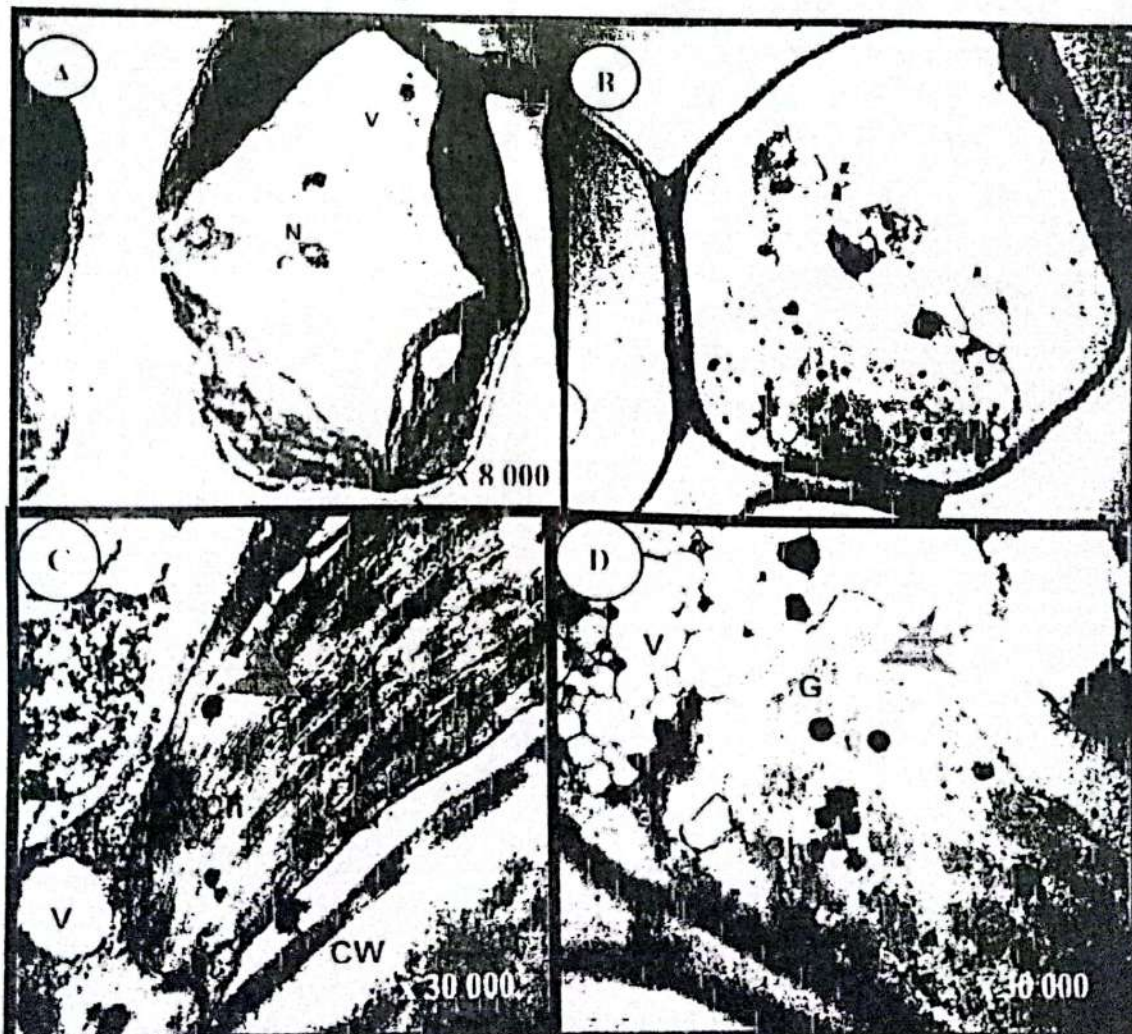
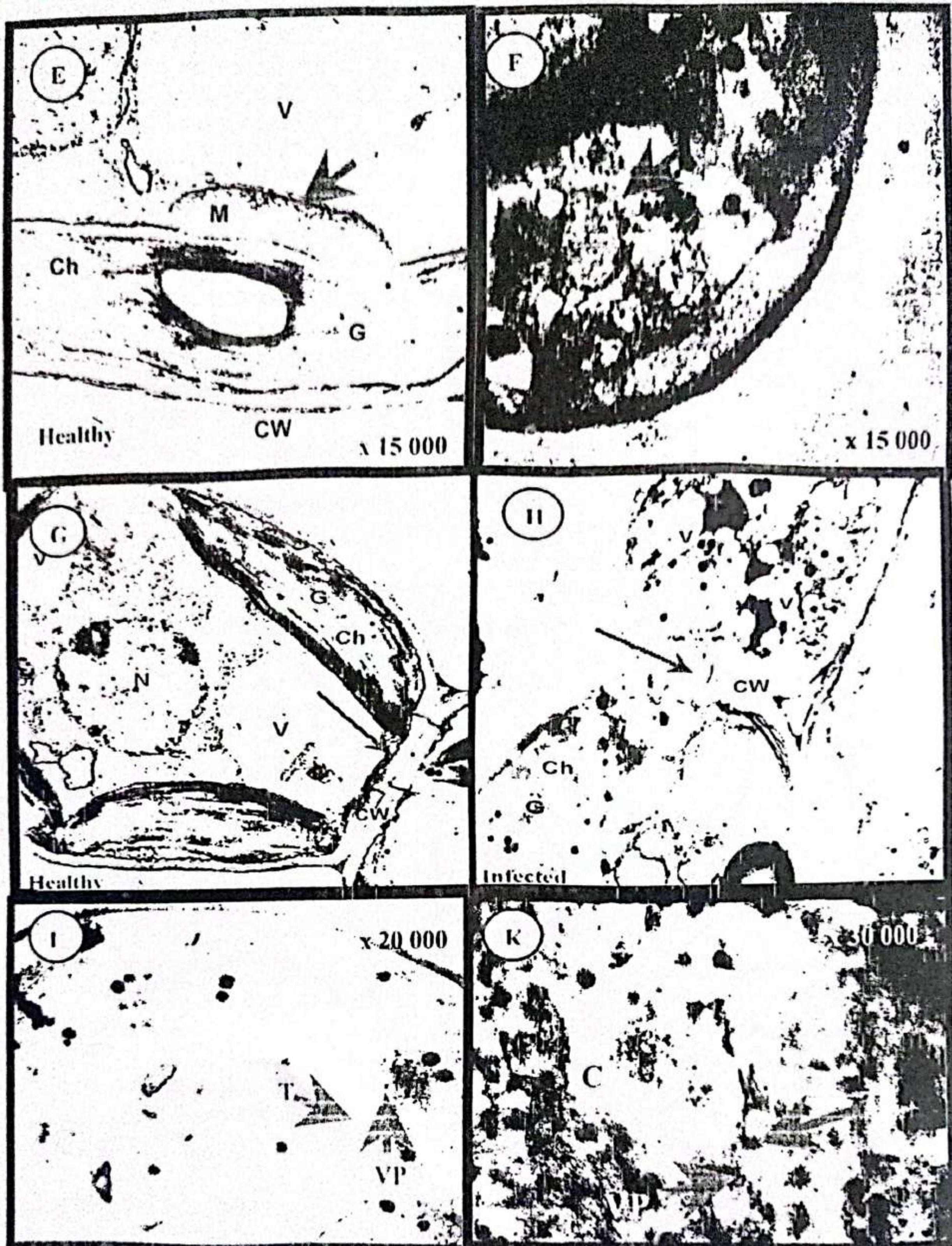



Fig. (6): Ultrathin section through leaves of peach cv. GF305:
Picture (A, C, E and G) Ultra thin sections of healthy leaves.
Picture (B) Ultrathin sections of infected leaves showing severe deformation in cytoplasm structure.
Picture (D) Ultrathin sections of infected leaves showing severe degeneration of chloroplast structure.




Picture (F): Ultrathin sections of infected leaves showing mitochondria appeared to be round than normal.

Picture (H): Ultrathin sections of infected leaves showing an outgrowth (O) of plasmodesmata containing a tubular structure with the virus particles in a cell of spongy paranchyma. Also, a Tubular structure with virus particles in plasmodesmata between adjacent mesophyll cells. CW, Cell wall; V, Vacuole; N, Nucleus; Ch, Chloroplast; G, Grana; M, Mitochondria.

Picture (I and K): Ultrathin sections of infected leaves showing a tubules structure (T) with virus particles (VP) in the Cytoplasm (C) of a mesophyll cell.

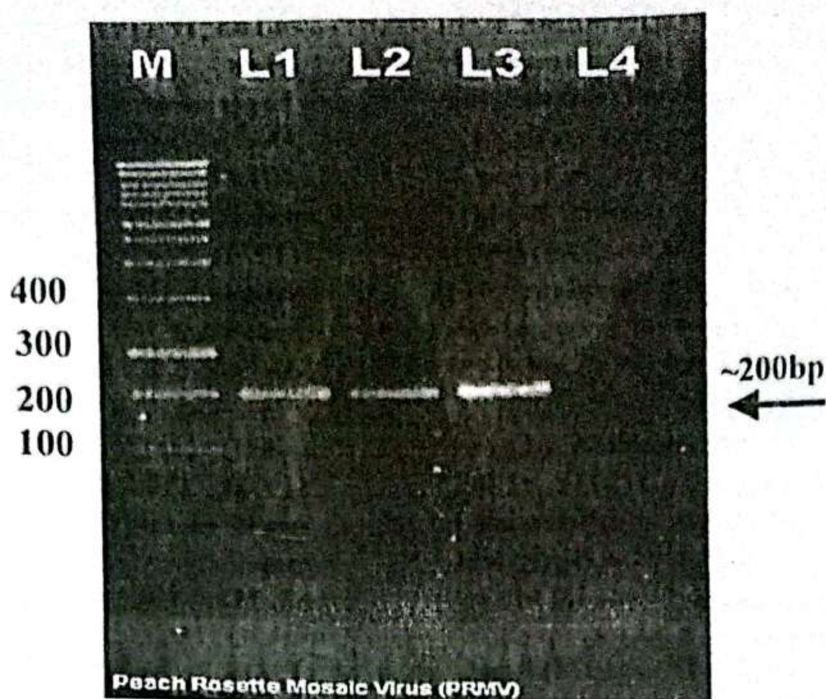



Fig. (7) : Electrophoresis analysis of RT-PCR products of PRMV. M; DNA molecular marker. (Lane 1) positive control for PRMV (Lane2and 3) infected samples while (Lane 4) showing the healthy control.

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