SEROLOGICAL STUDIES ON SOME PLANT VIRUSES

THESIS

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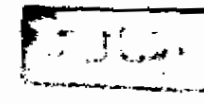

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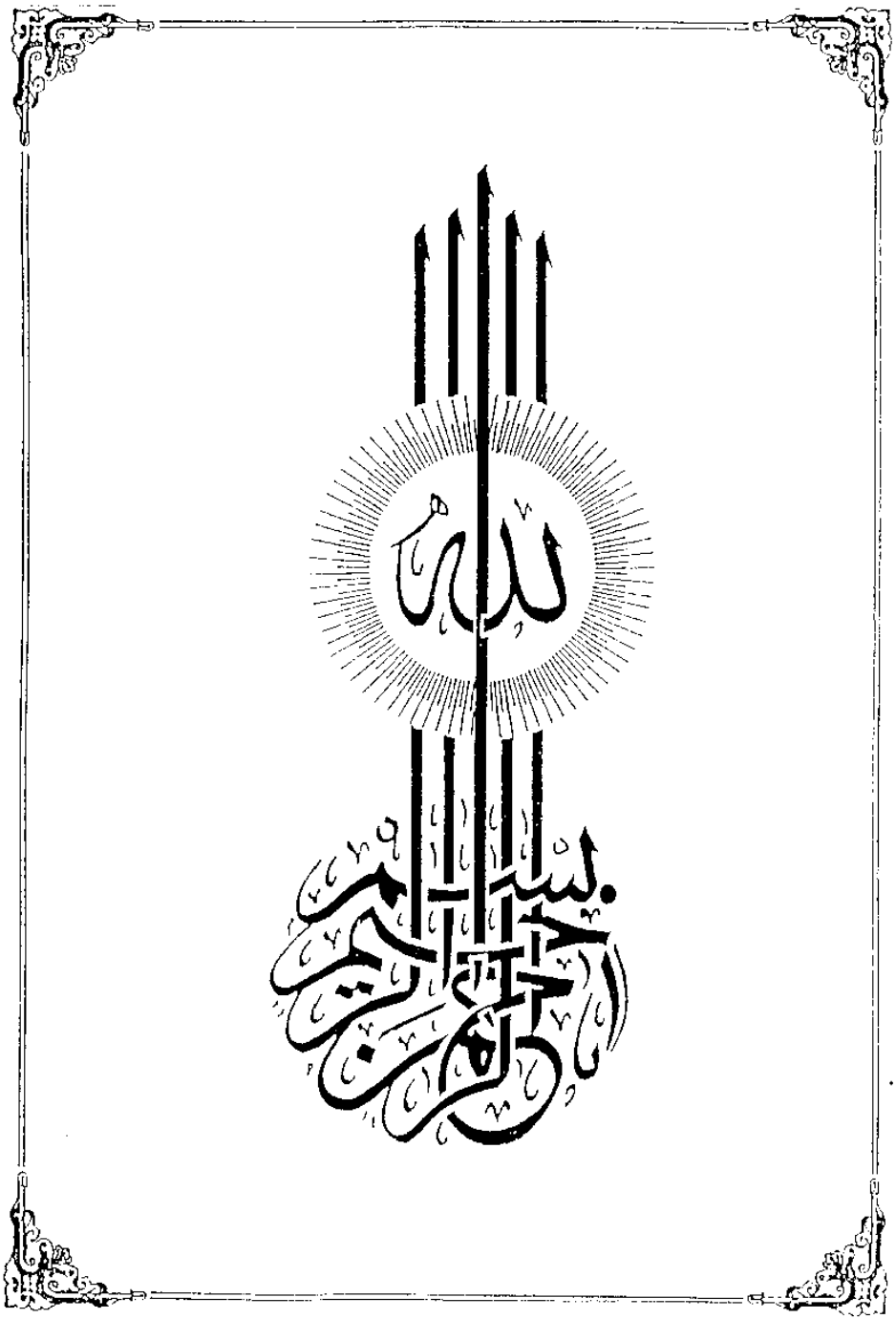

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INTRODUCTION

Most, if not all economically important crop plants in our country (Egypt) may become infected with viruses. In most cases the viruses cause a reduction in yield or quality of the infected crop, but the extent of the economic loss can vary greately. Also viruses are being increasingly recognized as a major constraint to crop improvements.

The economic importance of plant viruses, seen not only in the value of the crop losses they cause , but in the high cost of preventative or control measures required to avoid infection. Such measures include chemical spray to control insect vectors, the provision of virus free seed to prevent seed borne disease, breading for disease resistance and certification schemes to provide healthy planting stock for vegetatively propagated crops.

The main line of thought in working out the structure of this work , was to emphasize viruses as factors infecting the economically important vegetable crops in our country (tomato and potato plants) causing a great loss in these crops.

Tobacco mosaic virus has been estimated to cause between 15-25% loss of yield in infected tomato crops (Broadbent, 1976). In addition potato virus X was estimated to have caused losses in Australia amounting to $ 1,750,000 per annum at 1941 values . (Blad and Norris 1941).

In this work serological studies increase our knowledge about the diversity of viruses, playing a major role in rapid and specific detection of viruses and improved diagnosis.

In addition serological tests were used to evaluate the effeciency of the produced antisera for both TMV and PVX viruses. Slide agglutination test used mainly for virus detection, liquid precipitin test, microprecipitin test and Ouchterlony test mainly used for the determination of the antiserum titer prepared for each virus. Rocket immunoelectrophoresis method used for virus detection and also indicating the antigenic specificity of the antigen against its specific antibodies . In addition immunoelectron microscopic detection of PVX and TMV was carried out in this work. This method (of combine serology and electron microscope) has been found helpful and sensitive for the detection of the two viruses.

Moreover, electron microscopic examination increase our knowledge about the shape and the size of PVX and TMV viruses. These studies help us to improve virus control.

REVIEW OF LITERATURE

1- HOST RANGE AND PHYSICAL PROPERTIES

A) tobacco mosaic virus (TMV)

Different strains of TMV produced local lesions on the inoculated leaves of *Nicotiana glutinosa* and *Datura stramonium* (Rappaport and Wildman, 1962) and Brack, 1964). In addition, Thomas (1966) added that when the leaves of *Datura stramoniumn* were inoculated with a common strain of TMV a necrotic local lesions developed after three days. Also TMV produced a wide range of mosaic symptoms on a great number of hosts. These symptoms differed in severity on tomato and tobacco plants that varies from one strain to another, (Sigel and Wildman, 1954; Golden and Vestrova, 1959; McRichie and Alexander 1963). Moreover Rao and Reddy (1971) found that a new strain of TMV caused necrotic local lesions on *Nicotiana glutinosa*, *Nicotiana rustica* and *Nicotiana* sylvestris, The new strain was designated as the necrotic strain. Also Yoichi and Chiaki (1971) reported that when the detached leaves of bean were inoculated with TMV, a great number of local lesions are developed. Vela (1972) isolated a strain of TMV that produced mosaic, stunting and local lesions in *Nicotiana tabacum* var white burley, *Nicotiana* sylvestris, *Nicotiana rustica* and *Chenopodium* spp.,

and systemic mosaic followed by vein and stem necrosis in *Petunia hybrida*. In addition Allam and Abou El-Nasr (1971) concluded that necrotic local lesions are produced on *Nicotiana glutinosa*, *Datura stramonium*, *Datnra metel*, *Nicotiana tabacum* var white burley , *Nicotiana rustica* and *Solanum* melongena, while plants of *Gomphorena globosa* and *Petunia hybrida* reacted with local necrotic lesions accompanied by systemic mosaic through inoculation with eight TMV strains. Fujisawa et al. (1982) were able to isolate a strain of TMV from field grown spinach with mosaic symptoms and infected many cruciferae. The isolated virus was identified as a crucifer strain of TMV. In addition Oshima, et al.(1982) isolated Lycopersicon fruit deforming strain (LFD-ST) of TMV from an immature fruit of a streak-diseased tomato plants with convex irregular green rings, 1-2 cm diam., on the surface. Also this strain caused dark brown necrotic streaks along the veins and the undersides of the leaves and later on petioles and stem. The leaves developed interveinal necrotic spots and sometimes mosaic sysmptoms. This strain of TMV caused necrotic local lesions on the inoculated leaves of several tobacco cultivars, *Nicotiana* spp., *Petunia* and *Chenopodium amaranticolor* but no symptoms were produced on *Phaseolous vulgaris*. In addition, Verma and

Kumar (1982) isolated a strain of TMV that caused a yellow mottle disease of tomato. This virus was sap transmissible to members of the Amaranthaceae, Chenopodiaceae, and Solanaceae. Marchoux et al. (1983) showed a differential reaction of five *Petunia* spp. to different strains of TMV. They added that, inoculation of *P. axillaris* , *P inflata*, *P. parodii* and *P. violace* gave hypersensitive reaction or systemic infection. The lines showed differential behaviour towards a given TMV strain while the different strains induced specific reaction in the given lines. Li et al. (1983) isolated a crucifer strain of TMV isolated from garlic with mosaic symptoms. Moreover, El Ahdal et al. (1984) used *Nicotiana glutinsa*, *Nicotiana rustica*, *Nicotiana tabacum* var white burley, *Nicotiana sylvestris*, *Nicotiana paniculata* and *Chenopodium amaranticolor* as differential hosts for two strains of TMV. In addition Plessis (1983) isolated a strain of TMV from grapeveins showing leaf roll symptoms. Besedina, (1984) reported that about 150 isolates of TMV obtained from tomato plants were classified into 4 groups according to symptoms shown by indicator species. These were Nicotiana glutinosa, *Nicotiana sylvestris*, two *Nicotiana tabacum* cultivars, *Datura stramonium*, *Gomphorena globosa* and *lycopersicon esculentium*. Moreover Tarku, and Tolin, (1985) stated that soyabean was

infected by TMV and the symptoms was vein clearing with mild mosaic and the isolate was identified as a new strain of TMV on the basis of host range, symptomatology, light and electron microscopy and serological tests. Baez and Blanco (1985) reported that on inoculation of two tomato varieties with TMV, the weight and quality of fruits were considerably reduced and the quality was very poor. Taraku and Tolin (1988) isolated TMV from plants of soya bean with vein clearing and mild mosaic symptoms . In addition Sun,(1985) isolated TMV from *Paulowria tomentosa* with rolled leaves and interveinal yellowing. Also Randeles (1986) showed that *Echium plantogineum* L. infected locally with U1 strain of TMV and systemically by U2 strain by mechanical inoculation. Nicoud et al. (1987) reported that the hypersensitive reaction of *Petunia* axillaris cv. to strain SM_1 of TMV was accompained by the production of four unusual proteins in the tissue around the necrotic local lesions. In addition Nagai et al.(1987) isolated a new strain of TMV (TMV-U_1) from sweet pepper with mosaic symptoms and local lesion respectively . In addition Sherwood et al. (1988) reported that *Capsicum annuum* showed mild stunting and leaves with mild mosaic or severe stunting and severely chlorotic to necrotic symptoms. They added that TMV and PVY were found in plants with milder and severe symptoms.

Many investigators studied the physical properties of TMV. Allam and Abou El-Nasr (1971) found that the thermal inactivation point of eight TMV strains range between 80 °C to 90 °C, dilution end point range between 10^{-4} to 10^{-6} and the ageing in vitro range between 546 to 598 days . In addition Gibbs and Harrison (1976) reperted that thermal inactivation point of some TMV-strains was 95 °C and longivity in vitro more than one year. Verma and kumar (1982) stated that the new isolated TMV strain was transmitted by sap inoculation and the sap was infective at 10^{-7} but not 10^{-8} dilution. Thermal inativation point after 10 min was 85 °C but not 90 °C and after 45 but not 50 days storage at 25-35 °C. In addition Walkey (1985) showed that the thermal inactivation point of TMV range from 85 °C to 90 °C, dilution end point was 10^{-6} and the virus can survive for months at 20°C to a year at 0-2 °C.

B) Potato virus X (PVX): Servere mosaic symptoms are produced on *Datura tatula* when infected with PVX (Hooker and Benson, 1960). In addition, Smith (1972) stated that different potato varieties, King Edward, Arran Crest and Epicure infected with PVX produced acute necrosis of the growing points and usually died. Also Bhardwaj and Singh

(1976) identified three strains of PVX (X4, X5 and X6) isolated from potato seed stocks on the basis of their distinct reactions in *Capsicum pendulum* and certain other indicator plants. Moreover , Talens (1979) isolated a new ring spot strain of PVX that caused ring spots systemic line pattern and necrotic streaks on *Capsicum annuum*, *C. frutescens*, *Datura stramonium*, tomato and *Nicotiana rustica*, and necrotic local lesions on *Chenopodium amaranticolor*. Moreira, et al (1980) showed that a strain of PVX, caused necrotic local lesions, mild mosaic and systemic chlorotic blotching of symptom infection in 16 wild potato species. Also Sateri, et al (1980) found that plants of *Zinnia elegans* when infected with PVX produce a mosaic symptom and a reduction in plant and leaf size. Leiser and Richter (1980) differentiated three strains of PVX on the basis of their symptoms on *Nicotiana rustica*. Fox (1982) isolated an unusual strain of PVX in the potato cv. King Edward. Singh (1982) reported that *Physalis angulata* causes a systemic mottling upon infection with PVX. In addition, Klein et al. (1982) found that PVX gave local lesions on *Gomphorena globosa*. Also Rodriguez, et al. (1984) reported that, on the basis of host range, physical properties, serology and cross protection, the pathogen producing slight mosaic and yellowing on

Physalis ixocarpa was identified as astrain of PVX and the host range was confined to *Amaranthaceae*, *Chenopodiaceae* and *Solonaceae*. Moreover, Harvath (1985) stated that in inoculation experiments, *Physalis lanceifolia* was locally and systemically susceptible to TMV and PVX viruses. These results are in *agreement* with those obtained with other *Physalis* spp. In addition, Kaczmarek (1985) found new hosts for PVX from weeds of potato and from other field crops and natural habitats. These hosts were, *Urtrica cannabina*, *Rumex acetocella*, *C. arvense*, *Chelidonium majus*, *Fumaria officinalis*, *Papaver rhoeas*, *Dussurania sophica*, *Impatiens balsamina I. noli-tangere*, *Convolvulus tricolor*, *Anchusa officinalis*, *Linaria repens*, and *Verbascum thapsiphorme*. Vicenta et al. (1987) stated that *Gomphorena globosa* is a good local lesion host for PVX studies.

From eighteen isolates of PVX, Ladeburge et al (1950) stated that these isolates retained infection at dilutions of 1:500,000 but dilution of sap at 1;1000,000 resulted in complete loss of infectivity and the thermal inactivation point was 73 °C.

In addition, Hooker and Benson (1960) found that the dilution end point of PVX clarified virus from infected