SERIOLOGICAL AND BIOLOGICAL DIAGNOSIS OF SOME PLANT VIRUSES

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THESIS

Submitted

In Partial Fulfilment for the

Requirements of the Ph. D. Degree

Ain Shams University

Faculty of Agriculture

Cairo - Egypt

1975

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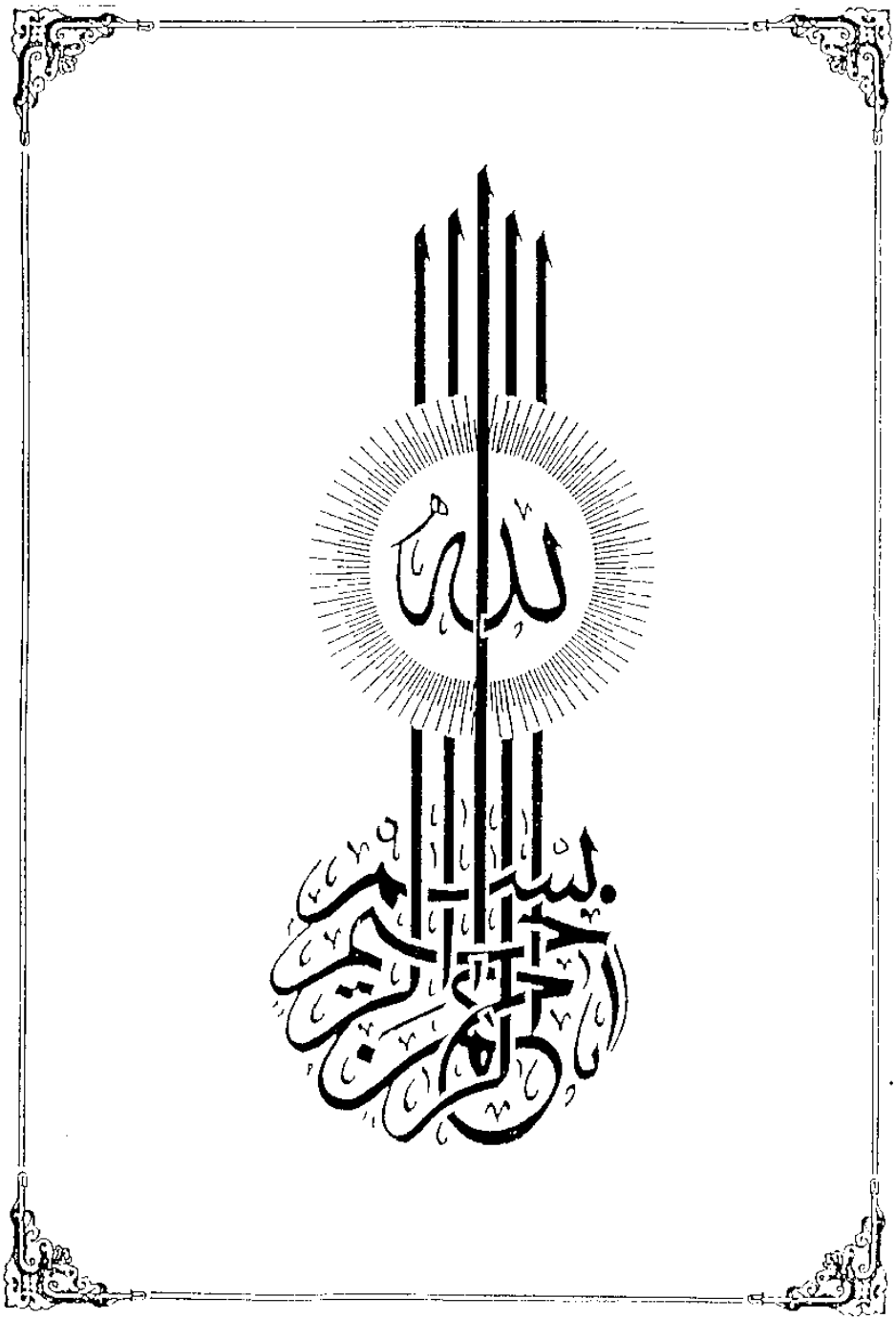

C O N T E N T S

ACKNOWLEDGEMENTS

The authoress, feeling sincerely grateful, has nothing to offer in return but to express her hearty gratitude and thankfulness for both Prof.Dr. E.K. Allam of 'Plant Virology', Faculty of Agriculture, Ain Shams University and Prof.Dr. S.El-Din Taha of : Microbiology Ain Shams University for suggesting the topic in question, supervision and incessant guidance throughout the research work.

It is the authoress' pleasant duty to express her esteem and indebtedness to all, who offered a helping hand and contributed in bringing this work to its present state which I do hope would give satisfaction.

INTRODUCTION

Tomato, Lycopersicon esculentum Mill and potato Solanum tuberosum L. are important economic crops in A.R.E. both for local consumption and export.

The cultivated area with tomato has increased from 234278 feddans in 1968 to 247112 in 1971. The annual production was 1495815 and 1636896 tons respectively in the same years.

A number of pests especially viruses inflict severe losses on tomato yield annually and TMV is perhaps the most contributing factor that affects both tomato yield quantity and quality.

The cultivated area with potato has increased from 53327 feddans in 1966 to 65623 in 1971. The annual production was 324083 and 1636896 tons respectively.

There are two main factors that should be taken into consideration concerning potato cultivation in the A.R.E. The first factor is that the mean yield of the feddan in the Nile cultivation may amount to 30% - 70% from the mean yield of the feddan in summer cultivation. As an illustration the feddan mean yield in 1964 was 3.3 tons in the

summer cultivation while it was 5.9 tons in the Nile cultivation. The second factor lies in the fact that the annual importation of the potato seed necessary for summer cultivation needs hard currency which increases continuously. It amounted about £ 1.5 millions in 1973. The virus infection is the fundamental reason for the importation of potato seeds and the reduction in the Nile yield.

This research work aims at the serological and biological diagnosis of tobacco mosaic virus and potato virus X. So, it aims at manifesting the difference between the virus infected and healthy plants in view of the following :

(a) The external symptoms

(b) Serological tests

(c) Chemical analysis

(d) The given yield

Trials were carried out to prepare antisera with high titer and specificity for serodiagnosis

REVIEW OF LITERATURE

I. DIAGNOSIS OF VIRUS IN INFECTED PLANTS

A. Isolation of Virus Nucleoprotein from Infected Plants:

Many methods have been described for the isolation and purification of plant viruses (Steere, 1959), most of which involve multistep procedure to remove contaminants by techniques such as :

1. Salt Precipitation

Precipitation of TMV by ammonium sulphate was reported by Vinson (1927, and 1938), Stanley (1935, 1936), Pfankuch & Kausche (1938); Van Koot (1940); Algera et al (1947); Nikiforova (1959); Vincent et al (1961); Allam (1961); Steere & Aokers (1962); Taniguchi & Fakeshi (1964); and El Afifi (1970).

Some other salts were used to salt out TMV from the juice of diseased plants, such as aluminum sulphate (Allard, 1916); lead acetate (Vinson & Gildehaus, 1932; and Chester, 1935a); and anhydrous sodiumsulphate (Vinson et al 1939).

The use of ammonium sulphate in PVX precipitation was recorded by Bawden & Pirie (1938); Pfankuch & Kausche

(1938); Rischkov & Gromyko (1938); Vinson et al (1939); Bawden & Kleczkowski (1948); Gorodskya (1950); Pirie (1956); Panjan et al (1963); Sheldko (1964); and El Afifi (1970).

2. Differential and Density Gradient Centrifugation

Differential centrifugation has been widely and successfully used for purification different viruses, alfalfa mosaic virus (Ross, 1941, and Bancroft et al., 1960a); Virus in cattleya orchids (Zaitlin & Schechtman, 1954); tobacco ring spot virus (Steere, 1956); Virus causing cherry yellows (Willison et al., 1956); Virus-like particles from woody plants (Thornberry, 1958), Sour Cherry necrotic ring spot (Fulton, 1959); wheat streak mosaic virus (Moorhead, 1959); white clover mosaic virus (Bancroft et al., 1960b); tomato black ring, rasberry ring spot, and arabis mosaic viruses (Harrison & Nixon, 1960); cucumber mosaic virus (Tomlinson et al. 1960, Scott, 1963, and Fremaine, 1966) barely strip mosaic virus (Kassanis & Slykhuis, 1960, Murayama & Yokoyama, 1962, and Nixon & Scott , 1966); brome mosaic virus (Hamilton, 1961, and Chiu Ren Jong & Gill, 1963), Cowpea mosaic virus (Agrawal, 1964); celery yellow vein virus (Hollings, 1965); a second

virus (Bancroft, 1962); tobacco ring spot (Corbett, 1961, and Stace-Smith et al., 1965); tomato spotted wilt (Black et al., 1963); barely yellow dwarf virus (Rochow & Brakke, 1964), Crowley et al (1965) obtained partially purified lettuce necratic yellows virus by differential centrifugation and rate zonal centrifugation in sucrase density gradients. The purified suspension infected N.glutinosa when diluted to 10^{-5} but not at 10^{-6}. Delgardo - Sanchez & Grogan (1966) purified PVY by differential and density gradient centrifugation, they obtained particles 684 mu in length. Izadpanah & Shepherd (1966); Corbett & Grant 1967; Guilermo & Galvez 1967; Galvez & Guillermo 1968; Ross (1967); Shephard & Grogan (1967); and Lee (1968); Fribourg & de Zoeten (1970); Kiriyama (1972); and Jones & Tolin (1972) used differential and density gradient centrifugation to purify pea enation mosaic virus, citrus variegation virus, rice tungro virus, soybean mosaic virus, western celery mosaic, wheat striate mosaic virus, potato virus A, cucumber mosaic virus, and maize dwarf mosaic virus respectively.

Concerning TMV, differential centrifugation was reported by Malkiel & Stanley (1947); Malkiel (1948); Watanebe & Kawade (1953); Wyad (1953); Rappaport & Siegel

(1955); Steere (1956); Takahashi et al (1958); Rappaport (1959), Dunin & Hitchborn (1965); Rees & Margaret (1965); Streeter & Gordan (1966); Brakke et al (1968); Yoshiaki & Takebe (1969); and Yuzo Nozu & Yamaura (1971).

Potato virus X particles have a tendency to aggregate to form large entangled masses that become insoluble jellies (Kleczkowskie Nixon, 1950). Reichmann (1959) reported purification of nonaggregated PVX by dialysis against sodium citrate and differential ultracentrifugation of the dialyzate. Corbett (1961) obtained non-aggregated particles by rate zonal density gradient centrifugation. Wright & Hardy (1961), and Chiko & Guthrie (1969) isolated PVX by high and low speed centrifugation, suspended the pellet in 0.005 M sodium citrate after each high speed centrifuation. Shepard & Secor (1969), Shepard & Shalla (1970, and 1972), and Shepard & Secor (1972) purified PVX by differential centrifugation. After a low-speed centrifugation, PVX was purified by a slight modification of the polyethylene glycol precipitation method devised by Gooding & Hebert (1967).

3. Zone Electrophoresis

This technique was used successfully in purifying TMV & PVX (Townsley, 1958); Watermelon mosaic virus

Concerning TMV, Hebert (1963) found that most of TMV was precipitated by 4% PEG in 0.1 M NaCl and 2% PEG in 0.3 M NaCl. He found that this method is suitable for purification of both rod-shaped and spherical viruses. Gooding & Hebert (1967) described a modification method of the PEG technique reported by Hebert (1963). They found that approximately 1 gm of TMV was recovered per 1,000 gm of systemically infected tabacum 'Hicks' leaves. Based on infectivity, less than 2% of the virus present in the initial extract was lost in the purification process.

The use of PEG precipitation for PVX purification was reported by Jermoljev & Alberechtova (1969), and Separd & Secor (1969).

5. Column Chromatography

Column chromatography has been widely used for isolation virus nucleoprotein. Cellulose ion exchangers were sucessfully applied in the purification of a number of animal viruses. Klemperer & Pereira (1959), and Haruna et al. (1961) isolated adenovirus by means of diethylamino-ethyl (DEAE) cellulose. Creaser & Taussing (1957), and Taussing & Creaser (1957) studied the behavior of a bacteriophage in relation to EC.E.L. - cellulose.

With plant viruses, partly purified preparations have been used in chromatography. Cochran et al (1957) sucessfully purified TMV with a cellulose ion exchanger after removal of other nucleoproteins. Levin (1958), using a similar technique, isolated PVX and TMV but did not obtain a complete separation of the various virus strains. Von Tavel (1959) separated TMV nucleoproteins on EC-TEOLA cellulose. He found that after three chromatographic runs about 10% of the contaminants had been removed. Osten (1958) purified TMV and PVX from other contaminants by step wise elution from the anion exchanger DEAE-cellulose. Toyoda et al. (1965) described a procedure for purifying rice dwarf virus by means of a DEAE cellulose column. Khuk & Kishko (1966) found that the PVX antigen after gel filtration through sephadex G-25 and purification on DEAE-cellulose could be seen clearly without non-specific fluorescence.

a. Cellulose Columns With Various Mixtures of Aqueous Polymers

This has been used for the complete separation of virus nucleoproteins. Albertsson (1960) in his studies on the partion of biological particles in aqueous polymer two phase system has developed methods which are of general

utility for the isolation of viruses. Using this type of system, Philipson et al (1960) purified echoviruses, adenoviruses, influenza virus, and T_2-phage without, apparently, any deleterious effects on their infectivities. Kamienska - Zyh, (1966) isolated PVY without aggregation by means of a cellulose column, with 4% PEG, 1% NaCl, and phosphate-citrate buffer. Banttarie & Zeyen (1969) purified oat dwarf virus using cellulose column chromatography with solutions containing PEG and NaCl as solvents, then sucrose density gradient centrifugation. Sivers & Shelud' Ko (1970) completely separated PVX containing fractions from the normal components with anion exchange TEAE-cellulose in a hydroxyl form or sephadex G-200. The purified virus particles were about the size typical of PVX. Kiriyama (1972) purified CMV using ion exchange cellulose.

Concerning CMV & PVX purification by cellulose-PEG system, Venekamp & Mosch (1963) used calcium phosphate columns and DEAE cellulose columns in an attempt to isolate PVX. They developed a new chromatographic method using cellulose with the aid of various mixtures of PEG in NaCl. But clarification by shaking with chloroform using the technique of Schneider (1963) resulted in a considerable

loss of active virus. Venekamp & Mosch (1964a) found a suitable method for the separation of TMV from chloroplasts prior to subsequent virus chromatographic purification. Purified virus was eluted from the column by the step wise addition of solvents carrying decreasing concentrations of PEG and NaCl. Venekamp & Mosch (1964b) developed a simple chromatographic separation of plant viruses (TMV, PVX, PVY, white clover mosaic) from chloroplast materials using cellulose columns with mixtures of 0.05% dextran, 4.5% glucose, 0.004 M $MgCl_2$, 0.01 M phosphate buffer pH-7 and varying concentration of PEG and NaCl as solvents. Venekamp & Mosch (1964c) using the same previous system for purification PVX, PVY, TMV, and potato stem mottle virus.

These purification methods achieved the complete separation of chloroplasts along with separation of most of the other unwanted impurities from the virus. High recovery of highly infectious virus was obtained. Leberman (1966) used PEG-dextran sulphate system in purifying turnip crinkle virus and turnip yellow mosaic, P_4 phage, and TMV. He found that isolation of viruses using water-soluble polymers have advantage over conventional methods.