COMPARATIVE STUDIES OF SOME METHODS FOR PREPARING ANTISERA FOR TOBACCO MOSAIC VIRUS AND POTATO VIRUS X

By

Sohair Ibrahim El-Afifi
B.Sc. (Agric.)
Ain Shams Univ., 1966

Thesis
Submitted in Partial Fulfilment of
the Requirements for the Degree of
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has been Approved by :

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A C K N O W L E DGE M E N T

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[illegible] ments of the people 13000 tons of tobacco leaves are annually imported.

However, in a very limited area in State farms tobacco is cultivated for research purposes. A great number of viruses affect tobacco plant in the U.A.R. The ravage caused by these viruses is not confined to tobacco plant only but they attack other economic vegetable crops the most important of which is tomato. This crop serves for local consumption and export. Its annual production was 1495815 tons in 1968. The exported amount of tomato fruits in 1968 was 379 tons.

Concerning potato the annual production was 472030 tons in 1968. The imported amount of potato seeds in 1968 was 23551,200 tons while the exported amount was 29793 tons in the same year.

Nowadays, the U.A.R. government is making strenuous efforts to increase the export of potato and tomato fruits for consumption and decrease the tonnage of imported potato because of the need for foreign currency.

A [illegible] growth, yield, and quality of both these

[illegible] virus isolate.

[illegible] was designed to purify antisera, in high activity and specifity for tobacco mosaic virus (TMV), and potato virus X (PVX). The highly active antisera are used widely in virus identification, as well as in selecting virus - free seed. To obtain an active antisera it is necessary to purify the antigen and accumulate it. Different methods of extraction and purification TMV and PVX were compared. This work also include comparisons between immunization methods for antisera production.

REVIEW OF LITERATURE

I. Virus Purification :

It is well-known that the model purification procedure includes clarification of infected sap, precipitation of virus as well as the removal of bound non-virus components.

Numerous methods of virus purification according to the steps mentioned above have been recorded by so many workers.

A. Clarification of infected sap :

In an attempt to purify plant viruses for the first time, it is a matter of prime importance to clarify virus extracts from other plant contaminants. This is done to make sure that purification steps are carried out with the appropriate fractions containing high percentage of the virus. It is clear from several clarification methods that each one could be used for many viruses. These methods include centrifugation and filtration, heating and freezing, acidification, the use of either hydrated calcium phosphate or organic solvents.

[illegible] by removal of plant cell debris and virus particles. Vinson (1927) clarified TMV from infected tobacco juice by low speed centrifugation. McClement (1934) obtained clear-straw-coloured supernatant of PVX from diseased tobacco plants by cooling the juice to 0°C. Carbon dioxide was passed through the mixture at 0°C for 30 min., then centrifuged at 3,000 rpm for 15 minutes. Bagnall et al. (1959) clarified PVM, PVS and carnation latent virus from other plant contaminants by centrifugation for 10 min. at 1000G, followed by shaking with half its volume of peroxide-free ether, then recentrifuged. Later on it was shaken with a similar volume of carbon tetrachloride and reclarified. Fulton (1959) stated that juices of sour cherry necrotic ring spot and prune dwarf viruses were clarified by the use of 0.2 M phosphate buffer (pH 7.0) containing 0.01 M sodium diethyldithiocarbonate, and 0.02 M sodium thioglycolate. Low speed centrifugation was carried out and the supernatant was mixed with hydrated calcium, then centrifuged. Steere (1957) obtained clarified TMV juice by centrifugation at 1,000 to 1, 000 x for 15 min. In studies with white clover mosaic, Bancroft et al. (1960)

[illegible] clarified extracts containing clover
[illegible] virus by centrifuging for [illegible] rpm, after which the supernatant was centrifuged for 30 min. at 10,000 rpm. Bancroft (1962) used centrifugation at 10,000 rpm for 10 min. to clarify bean pod mottle virus extract. Brakke (1962) obtained clarified barley strip mosaic virus juice by centrifugation at 8000 rpm for 15 minutes. Recentrifuged after the addition of 0.2 M Na_2HPO_4 and $CaCl_2$. Corbett and Roberts (1962), and Leberman (1966) used low-speed centrifugation and filtration through celite or activated charcoal to clarify juices of tobacco ring spot and tobacco mosaic viruses. Black et al. (1963) clarified extracts containing tomato spotted wilt virus by centrifugation at 3000 - 5000 rpm for 30 min. Gibbs et al. (1963 a) removed solids from extracts containing barley strip mosaic and lychnis ring spot viruses by filtration through muslin, followed by low speed centrifugation. Hebert (1963) obtained clarified TMV juice by centrifuging the juice of tobacco plants at low speed ([illegible] rpm for 10 min.), heating the supernatant at 60°C for 10 min., then recentrifuging at low speed. Macleod and Markham (1963), Vonekamp

[illegible]

[illegible] yellows [illegible] lettuce necrotic [illegible] viruses respectively, obtained clarified juices [illegible] low speed centrifugation. [illegible]winiarski *et al.* (196[illegible]) were able to clarify PVY from infected leona potato by the use of 50% aqueous alcohol (10 : 3-4), then chlorophyll was separated by centrifugation at 45000 rpm for 10 min.

An alternative means to centrifugal clarification is filtration by filter paper or other materials. This was recommended by various investigators. Filter paper was recorded by Rischkov and Gromyko (1938), Steere (1959)& Corbett and Sisler (1964) to clarify TMV and PVX extracts.

Diatomaceous earth, celite, charcoal, and bentonite were also applied for preliminarly TMV clarification (Stanley, 1936a;Van Koot, 1940; Steere, 1963 ; Corbett and and Sisler, 1964; and Dounin and Kitchborn, 196[illegible]), for lettuce necrotic yellows virus (Mclean and Francki, 1967), and for apple chlorotic leaf spot (Saksena and Mink, 196[illegible]).

[illegible]. Organic solvents :

Many investigators made use of organic solvents such as n-butanol, chloroform, and acetone for initial

[illegible] reported that chloroform [illegible] Stanbury and Deusche (1961), Allam (1961), and Venekamp and Bosch (1963) used chloroform to clarify the TMV infected extract. In most of these procedures the emulsion was centrifuged, and left to stand for 10 - 20 hours at low temperature. Schneider (1953) reported that chloroform was used to denature non-infective materials present in the juice of tobacco infected with TMV, and the emulsion was broken by centrifugation. Cech (1962) obtained clarified TMV sap from freshly samsun tobacco leaves by centrifugation for 10 min. at 10,000 g. The supernatant was shaken with chloroform (10 : 1 w/w), and then recentrifuged. Chloroform was also used to separate PVX from host components (Venekamp and Bosch, 1963; Sheldko, 1964; and Chiko and Guthrie, 1968). The latter investigator centrifuged the resulting suspension for 15 min. at 11,000g.

[illegible] (1964 b) clarified extracts containing lucerne mosaic virus using chloroform. The emulsion was centrifuged at [illegible] for 10 min., stored overnight at room temperature, and then centrifuged at 5,000 g for 15 min. Scott (1963) homogenized tissues infected with CMV-Y by 0.5 M citrate buffer pH 6.5 (containing 0.1%

[illegible] potato yellow dwarf virus using chloroform, then [illegible] centrifuged at [illegible] for 15 minutes. Rochow and [illegible] recommended the use of chloroform and charcoal for the clarification of barley yellow dwarf and barley strip mosaic viruses. They recorded that this procedure removed effectively green and yellowish brown pigments.

N-butanol was also employed for clarification of CMV (Tomlinson *et al.*, 1960), TMV (Hebert, 1963), and potato yellow dwarf virus (Whitcomb, 1965). Chloroform-n-butanol mixture was also applied to the clarification of TMV (Steere, 1956, 1959), tomato black ring, rasberry ring spot, and arabis mosaic viruses (Harrison and Nixon, 1960), bean pod mottle virus (Bancroft, 1962), virus associated with tatter leaf of citrus (Semancik and Weathers, 1963), Cowpea mosaic virus (Agrawal, 1964), tobacco streak virus (Fink *et al.*, 1966) potato spindle tuber (Raymer and Diener, [illegible]), and potato leaf roll ([illegible] *et al.*, [illegible]).

[illegible]. Heating and freezing :

Most of the non-infective proteins were removed from the juice of infected plants by heat coagulation (Steere, [illegible]). Tomato bushy stunt virus was originally