UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION, PURIFICATION AND ANTISERUM PRODUCTION OF SWEET POTATO FEATHERY MOTTLE POTYVIRUS (SPFMV)

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By

KHAIRULMAZMI AHMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Agricultural Science

August 2003

DEDICATION

Special dedication to my beloved…

Father…

Mother….

&

To all my family members…

Lastly

To all my best friends

Abstract of thesis presented to Senate of Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Master of Agricultural Science

CHARACTERIZATION, PURIFICATION AND ANTISERUM PRODUCTION OF SWEET POTATO FEATHERY MOTTLE POTYVIRUS (SPFMV)

By

KHAIRULMAZMI BIN AHMAD

August 2003

Chairman: Inon Sulaiman, Ph.D.

Faculty: Agriculture

Characterization, purification and production of antiserum against sweet potato feathery mottle potyvirus (SPFMV) were carried out in this study. Characterization of the virus involved host range reaction, inclusion bodies determination, virion morphology and determination of coat protein molecular mass.

Initial serological detection of potyviruses infection was carried-out by Indirect ELISA using an Agdia® commercial kit for potyvirus on samples of sweet potato collected from major growing areas in Peninsular Malaysia, Sabah and Sarawak. Experimental results of this study showed, potyviruses are present in most of the

major sweet potato growing areas in Peninsular Malaysia and some areas in Sabah and Sarawak.

The SPFMV isolate used in this study was single probed by aphid and vectored to healthy *Ipomoea setosa* three times. The initial infection symptom on *I. setosa* was characterized by veinal chlorosis of actively growing leaves. In subsequent leaves, the veinal chlorosis was concentrated to major veins. Progressively the veinal chlorosis was restricted to midvein. Subsequent new foliar growth was symptomless.

Host reaction results showed, *I. nil* produced symptoms of faint vein clearing and crinkling after inoculation. Symptomless *I. purpurea* tested positive in Indirect ELISA, thus showing latent infection in this host. The rest of the indicator plants did not show any virus symptom and tested negative in the ELISA test. These results indicated that the virus isolate in this study has a narrow host range and restricted to the *Convolvulaceae* family only.

Cytoplasmic inclusions (CIs), a major characteristic of the poty group, were observed under light microscopy. In the infected cells, the CIs were stained dark blue (Azure A) and were usually located beside the plant cell nuclei.

Purification of SPFMV directly from infected sweet potato leaves by twice CsCl density gradient centrifugation yielded about 1.5 mg of virus per 100 g of fresh

tissue. The ratio of A_{260}/A_{280} nm was around 1.123 (without correction for light scattering). Electron microscopy revealed flexuous filamentous particles with a modal length of 750-849 nm. Protein analysis of denatured virus by SDS-PAGE revealed a major coat protein of 40 kDa.

Polyclonal antiserum in this study was produced against the purified local SPFMV isolate. The antiserum had a titer of 1:1536 in microprecipitin tests. The antibody reacted positively by DAS-ELISA with sap from SPFMV-field infected tissue and purified SPFMV preparation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains Pertanian

PENCIRIAN, PENULENAN DAN PENGHASILAN ANTISERUM TERHADAP SWEET POTATO FEATHERY MOTTLE POTYVIRUS (SPFMV)

Oleh

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Pencirian, penulenan dan penghasilan antiserum terhadap sweet potato feathery mottle potyvirus (SPFMV) telah dijalankan dalam kajian ini. Kajian pencirian melibatkan reaksi virus terhadap julat perumah, penentuan "inclusions bodies", morfologi virion dan penentuan berat molekul sarung-protein virus.

Ujian awal serologi terhadap jangkitan potyvirus telah dijalankan dengan menggunakan kaedah Indirect ELISA (Agdia® commercial kit for potyvirus) terhadap sampel-sampel keledek yang telah diambil daripada kawasan penanaman utama keledek di Semenanjung Malaysia, Sabah dan Sarawak. Keputusan ujian menunjukkan bahawa potyvirus telah menjangkiti kebanyakan

tanaman dikawasan penanaman utama keledek diSemenanjung Malaysia dan beberapa kawasan di Sabah dan Sarawak.

SPFMV dipencilkan melalui satu 'probe' vektor afid dan dijangkitkan kepada *Ipomoea setosa* sihat sebanyak tiga kali . Ciri simptom jangkitan awal pada *I. setosa,* adalah dengan pembentukan simptom urat jernih pada urat-urat daun yang masih aktif membentuk. Pada daun yang berikutnya, simptom urat jernih hanya tertumpu pada urat-urat utama daun. Seterusnya, simptom urat jernih hanya tertumpu pada urat tengah daun sahaja. Selepas itu, daun yang baru tidak lagi menunjukkan simptom.

Keputusan reaksi perumah, *I. nil* selepas diinokulasi adalah pembentukkan simptom urat jernih yang samar dan daun berkedut. Tiada simptom jangkitan pada perumah *I. purpurea*, tetapi mencatat keputusan positif dalam ujian Indirect ELISA. Ini menunjukkan berlakunya jangkitan terpendam pada perumah tersebut. Perumah yang lain tidak menunjukkan simptom dan ia juga mencatat keputusan negatif terhadap ujian Indirect ELISA. Keputusan ujikaji ini menunjukkan bahawa virus yang telah dipencilkan mempunyai julat perumah yang terhad kepada tanaman jenis *Convolvulaceae* sahaja.

"Cytoplasmic inclusion"(CIs) iaitu pencirian utama kumpulan poty, telah dikesan melalui mikroskop cahaya. Pada daun berpenyakit, CIs dapat dibezakan apabila

ia mengambil warna biru gelap (Azure A) dan biasanya terdapat berdekatan dengan nukleus sel tumbuhan.

Penulenan SPFMV secara langsung dari daun keledek berpenyakit dengan menggunakan penggunaan emparan "CsCl density gradient" sebanyak dua kali telah menghasilkan 1.5 mg virus daripada 100 g daun segar. Manakala nisbah A_{260}/A_{280} nm adalah pada sekitar 1.123 (tanpa pembetulan terhadap biasan cahaya). Keputusan mikroskop elektron pula menunjukkan partikal virus berbentuk rod melentur (flexuous rod) dengan panjang, 750-849 nm berdasarkan model panjang virus. Analisis protein virus menggunakan SDS-PAGE telah menghasilkan satu jaluran major sarung-protein yang berat molekulnya ialah 40 kDa.

Antiserum poliklonal dalam kajian ini telah dihasilkan dengan menggunakan SPFMV tempatan yang telah ditulenkan. Antiserum yang dihasilkan mempunyai titer sehingga 1:1536 pencairan dengan menggunakan ujian mikroprisipitin. Antibodi yang dihasilkan telah memberi reaksi positif terhadap sap dan daun yang dijangkiti oleh SPFMV dan juga SPFMV tulen dengan menggunakan ujian DAS-ELISA

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I certify that an Examination Committee met on 6 August 2003 to conduct the final examination of Khairulmazmi b. Ahmad on his Master of Agricultural Science thesis entitled "Characterization, Purification and Antiserum Production of Sweet Potato Feathery Mottle Potyvirus (SPFMV)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends the candidate be awarded the relevant degree. Members of the Examination Committee are follows:

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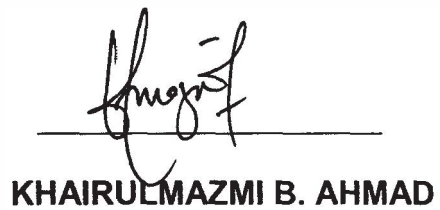
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that is has not been previously or concurrently submitted for any other degree at UPM or other institutions.



KHAIRULMAZMI B. AHMAD

Date: 30.11.2003

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Adjuvant	Substance injected with antigens (usually mixed with them but sometimes given prior to or after the antigen) which non-specifically enhances or modifies the immune response to that antigen.
Alkaline phosphatase	An enzyme which hydrolyses certain phosphate-containing compounds under alkaline conditions; commonly obtained from calf intestine mucosa
Antibody	A protein formed in blood serum in response to stimulation by an antigen. Antibodies are specific for their respective antigens, and antigens and antibodies are mutually attracted
Antigen	A substance which, when introduced into the biological environment of a vertebrate animal, leads to the formation of antibodies directed specifically against it. All immunogens are antigenic, but not all antigens are immunogenic.
Antiserum	Serum from any animal containing antibodies to a specified antigen.
APS	Ammonium peroxydisulphate
BIS	N. N' –Methylenbisacrylamide
BPB	Bromophenol blue
BSA	Bovine serum albumin
Chromatography	The separation of mixtures of chemicals, compounds, proteins, macro-molecules etc. into their constituents or components by preferential adsorption by a solid such as a column of cellulose, or by filter paper or by gel
Conjugate	The product of joining two or more dissimilar molecules by covalent bonds. In immunological contexts, one is usually a protein and the other either a hapten or a label such as fluorescent, ferritin, or enzyme

Dialysis	A procedure using a membrane to separate various components in solution in accordance with their ability to pass through the membrane
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay. An immunoassay system employing enzyme bound to antibody as the immunologic probe determining the extent of antigen-antibody reaction.
Immunogen	A substance that elicits an immune response when introduced into the tissues of an animal. To stimulate a response, immunogens must normally be foreign to the animal to which they are administered, of a molecular weight greater than 1,000, and of protein or polysaccharide nature.
Freund's Adjuvant.	A mixture of mineral oil and lanolin that enhances immune responses when emulsified with antigen for immunization. Freund's complete adjuvant includes killed mycobacteria; Freund's incomplete does not.
Immunoglobulin (Ig).	Serum globular glycoprotein. There are five classes of immunoglobulin, IgA, IgD, IgE, IgG, and IgM. IgG is the major immunoglobulin class in the serum of man and in most species from amphibians upwards.
kDa	Kilodalton
NC	Nitrocellulose membrane
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline-Tween
PVP	Polyvinylpryrrolidone
RNA	Ribonucleic acid

SPFMV	Sweet potato feathery mottle potyvirus
SPCSV	Sweet potato chlorotic stunt crinivirus
SPVD	Sweet potato virus disease
SDS	Sodium dodecyl sulfate
TEMED	N, N ,N, N, -Tetramethylethylendiamine
Tris-HCl	Tris-(hydroxymethyl)–aminomethane

CHAPTER 1

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is a dicotyledonous plant from the family *Convovulaceae.* The crop originated from Central or South America (Karyeija et al., 1998b). It is grown exclusively in tropical and subtropical regions with low input and can produce good yield under marginal conditions (Farayi et al., 1997). It has been planted in Malaysia for more than 100 years and more than 100 varieties are available (Saad, 1994). Traditionally, during the time of food shortage, sweet potato became useful substitutes of the staple food crop, i.e. rice (Tan and Saad, 1994). This crop has the potential to become an important supplier of starch to the other industries.

As with other crops, sweet potato is also susceptible to plant pathogens. Sweet potato diseases, especially those caused by viral infections have been identified as the second most important biotic component after insect infestations (Moyer and Salazar, 1989 ; Farayi et al., 1997). Previous studies indicated that there are five major potyviruses affecting sweet potato production. One of these viruses is the sweet potato feathery mottle potyvirus (SPFMV), which has been reported to infect sweet potato wherever it is grown (Moyer and Salazar, 1989).

SPFMV has been detected by observing symptom expression patterns in infected plants in the field, as well as on sensitive indicator plants like *Ipomoea setosa* and *I. nil* in biological assays (Farayi et al., 1997 and Karyeija et al, 1998a). However, SPFMV indexing has been hampered by absence of rapid, sensitive and reliable diagnostic tool for viral pathogens (Farayi et al., 1997). Hence, an improved detection system is needed to detect and refine the diagnosis of SPFMV.

Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) introduced by Clark and Adams, (1977) was able to solve the limitation of the biological assay. The ELISA techniques are known to be sensitive, cheaper and faster than biological assay. ELISA techniques have a higher level of sensitivity and reproducibility, yet employs relatively simple and inexpensive equipments (Clark and Adams, 1977). Results of preliminary studies on viral problem of sweet potato in Malaysia, indicated the presence of putative SPFMV (Inon et al., 1998).

Information on the epidemiology, symptomatology, host range, virus-vector relationship and molecular aspect of the putative SPFMV are still incomplete or lacking. Important information is needed to develop strategies for SPFMV management. Research on sweet potato development in Malaysia is still lacking. Only two institutions (MARDI and UPM) are working on sweet potato problems (Saad, 1994). Most of the work reported has been in the

area of plant improvement and breeding. This study was conducted with the following objectives:

1) To assess the spread of the SPFMV infection in sweet potato in the major sweet potato growing areas in Malaysia.
2) To characterize a local SPFMV isolate.
3) To purify the local SPFMV isolate.
4) To produce local antiserum against local SPFMV isolate.