Purification and Antiserum Production of Sweet Potato Feathery Mottle Potyvirus (SPFMV)

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Introduction

Sweet potato feathery mottle potyvirus (SPFMV) was first reported in *Ipomoea batatas* (L) in the USA. The range of symptoms associated with SPFMV infections are influenced by host genotype, the enviromental factors and the virus strains. Leaf symptoms of vein yellowing (vein feathering), chlorotic or purplish spots in some varieties are common symptoms associated with the virus (Cali and Moyer, 1981). SPFMV is a filamentous flexuous rod potyvirus with particle length of 810-850 nm. It is a non-persistent, aphid-transmissible virus and is not seed-borne (Moyer and Kennedy,1978). According to Moyer and Salazer (1989), SPFMV is distributed worldwide, infecting sweet potato wherever it is grown. Earlier studies carried out on the distribution of this virus in Malaysia, indicated that SPFMV is present in most of the sweet potato growing areas in Peninsula Malaysia, Sabah and Serawak (Knairulmazmi et al., 1999: Khairulmazmi et al., 2000a). Based on the finding that SPFMV is the most common and widespread virus on sweet potato in Malaysia, this study was carried out to purify and to produce antiserum against the local SPFMV.

Materials and Methods

The initial virus source was collected from infected sweet potato in UPM, Serdang, Selangor. It was three times isolated by aphid transmission to *Ipomoea setosa* and grafted to healthy sweet potato seedlings. Sweet potato that showed typical virus symptoms of vein feathering were propagated and maintained in insect-proof cages in the glass house.

Virus purification

Purification was carried out by the method of Cohen et al., (1988). Leaves of viral infected sweet potato were homogenized in 2 ml of 0.5 M borate buffer, pH8.0, containing 0.01 M EDTA, 1 ml chloroform,1 ml carbon tetrachloride and 1 µl of 2-mercaptoethanol per gram of leaves tissue. The mixture was centrifuged for 10 minutes at 10,000g and supernatant was mixed with 1% Triton X-100 for 2 hours at 4°C and centrifuged again for 2 hours at 95,000 after being layered on top of a 25% sucrose cushion. The pellets were resuspended in 0.05 M borate buffer pH 8.0 containing 0.001 M EDTA and left overnight at 4°C. The suspension was layered on a 10-40 % CsCl gradient column and centrifuged again on CsCl gradient as described above. The purified virus taken from CsCl densiy gradients was dialysed in buffer to remove CsCl. Absorbance spectra of purified virus were obtained using a spectrophotometer.

Antiserum preparation

Two intramuscular injections of purified virus (1 mg/ml) emulsified with an equal volume of Freund's adjuvant and one booster veinal injection were administered into a male New Zealand white rabbit at 2 weeks interval. Blood was collected one week after booster injection. The antiserum obtained was stored at –27°C. Microprecipitin test was conducted to determine antiserum titer.

Double-antibody sandwich, enzyme-linked immunosorbent assay (DAS-ELISA)

DAS-ELISA was conducted using the procedure of Clark and Adam, (1977). IgG was first purified by ammonium sulphate precipitation and anion exchange chromatography. The DAS-ELISA method includes coating an antibody to microplate wells followed by an incubation period of 2-4 hours at 37°C. This is followed by three times washing with PBS-Tween, then antigen was added and incubated overnight at 4°C. After washing with PBS-Tween, labelled

conjugate was added. Lastly, substrate (p-nitrophenyl phosphate) was added and after 60 minutes of hydrolysis at room temperature, the absorbencies were evaluated in a spectrophotometer (METERTECH-ELISA reader)at 405nm.

Results and Discussion

Virus purification

The predominant virus band in the CsCl density gradients was located approximately 20-21 mm from the bottom of the gradient tubes. The ultraviolet absorption spectrum had a maximum reading between 258-260 nm and a minimum between 235-242 nm with $A_{260}/A_{280\ ratio}$ 1.123 (without correction for light scattering). Based on the extinction coefficient $A^{1mg/ml}_{1cm,260\ nm}$ =2.5 commonly used for potyvirus, the average yield of purified was 1.5mg/100g of infected leaf tissue.

Serology

Microprecipitin test carried on the antiserum showed a titer of 1:1536 in the homologous and had a non-specific reaction of 1:16. To eliminate the prescence of non-specific reaction, cross absorption with partially purified healthy sap was conducted. Antiserum titer for SPFMV That had been reported were: 1:1024 (Moyer and Kennedy, (1978), 1:2024 and 1:4089 Cali and Moyer (1981, 1989)) respectively. DAS-ELISA results showed that the antiserum produced reacted positively with purified SPFMV and leaf sap of SPFMV infected plant collected from field and glass house.

Conclusions

Sweet potato feathery mottle potyvirus purified from infected sweet potato leaves by twice CsCl gradient centrifugations produced an average yield of 1.5mg/100g of leaf tissues. Antiserum produced against purified virus preparation had a titer of 1:1536 in microprecipitin tests. It reacted positively in DAS-ELISA with purified SPFMV and sap from infected leaves.

Benefits from the study

This study confirmed the present of sweet potato feathery mottle potyvirus (SPFMV) and it widespread in Peninsula Malaysia, Sabah and Serawak.. Managed to produce antiserum against the local strain of SPFMV (strain C), however, since it also contained non-specific antibodies, therefore there is a need to further improve the purification technique to eliminate plant protein which will induce non specific antibodies.

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals:

Nil

Project Publications in Conference Proceedings

1. Khairulmazmi A, Inon S, Zakaria S and Yaakob D. 2000a Potyvirus from diseased sweet potato collected from various locations in Malaysia. Plant Heath Conference 2000, 95-96.

2. Khairulmazmi A, Inon S, Zakaria S and Yaakob D. 2000b. Purification of sweet potato feathery mottle potyvirus (SPFMV) from infected sweet potato leaves and preliminary host reaction.23th Malaysian Microbiology Symposium.

3. Khairulmazmi A, Inon S, Zakaria S and Yaakob D. 2001. Some properties of sweet potato feathery mottle potyvirus (SPFMV) from infected sweet potato leaves and preliminary host reaction.24th Malaysian Microbiology Symposium.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Khairulmazmi Ahmad	Purification and Antiserum Production of Sweet Potato Feathery Mottle Potyvirus (SPFMV)	**Virology Tumbuhan**	**M. Agri. Sc.**	**2003**

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