Cucumber mosaic cucumovirus associated with kava plants showing symptoms of dieback disease in Fiji and Tonga


Abstract

Kava dieback disease was first recorded in 1935. While some of the symptoms were suggestive of a virus, no viruses have been detected in kava or any other member of the Piperaceae. Present results from dsRNA analyses, immuno-electron microscopy, ELISA, and transmission to Nicotiana tabacum and N. glutinosa showed that cucumber mosaic cucumovirus (CMV) was present in many dieback-affected plants. The virus was detected in 44% of kava samples showing a range of symptoms, and was most common in the cultivar Kava Leka Kula in which 61% of samples tested positive by immuno-electron microscopy.

Introduction

Kava (Piper methysticum (Forster), Family Piperaceae) is a perennial multi-stemmed shrub 2–3 m high. The stem bases and roots are harvested at 3–5 years, pulverised and mixed with water to produce an intoxicating beverage. It is therefore an important crop socially and economically in the Pacific islands where it is also known as ava, yaqona or grog.

The main constraint to kava production in Fiji and Tonga is a wilt/dieback disease (Brown and Minchinton 1989). The dieback was first recorded by Parnham (1935) in Fiji and has since been seen in Tonga, Vanuatu and Western Samoa. In Fiji it has been estimated that the disease causes annual crop losses of about 60%. Symptoms usually appear when plants are 1.5–2 years old (Brown and Minchinton 1989).

Surveys for kava diseases were conducted in 1988 in Fiji (Brown et al. 1989), Tonga (Minchinton et al. 1989), Vanuatu (Minchinton and Brown 1999a) and Western Samoa (Minchinton and Brown 1989b). The diseases and pests recorded were a leaf spot caused by Sphaerulina sp., black stem lesions associated with Colletotrichum sp., kava weevil borer (Elytroteles subtruncatus Fairm.) and root knot nematodes (Meloidogyne sp.). Control of the nematodes significantly lessened the incidence of wilt and stem rot (Brown and Minchinton 1989).

Although attempts to establish the cause of kava wilt in Fiji have been unsuccessful (Brown and Minchinton 1989), symptoms indicated that the disease might have been caused by a mycoplasma or a virus. To date no viruses or mycoplasmas have been recorded in any member of the Piperaceae.

In this paper we report the results of tests for viruses infecting wilt-diseased plants.

Methods

Mechanical inoculations were done by macerating leaf tissue from diseased plants in 0.063M phosphate buffer, pH 8.0 containing 0.25% (w/v) Na2SO4, rubbing the extracts onto leaves of the indicator species and then immediately washing the inoculated leaves with water.

Negative stains of plant sap were done by crushing a small leaf piece in 2% ammonium molybdate, floating an electron microscope grid on the extract for 10 sec, draining the grid by touching the edge to a piece of filter paper and drying before examination in the electron microscope.

Immuno-electron microscopy (IEM) was performed as described by Pares and Whitecross (1983) using antiserum prepared against a New South Wales isolate of cucumber mosaic cucumovirus (CMV) or alfalfa mosaic virus (AMV). The latter was kindly supplied by the late Dr R.I.B. Franki, Waite Institute, Adelaide. The virions were stabilised by macerating tissue in 0.05M Na2HPO4 containing 0.05% (v/v) thioglycollic acid, 0.05% (w/v) sodium diethyldithiocarbamate, and 0.5% (v/v) formaldehyde. Virus incubation time on the antiserum-coated grids was 4 h.

ELISA was performed as described by Clark and Adams (1977) using an anti-CMV antiserum supplied by Ms E. Alberts. The sample buffer was the same as that used for IEM as it has proved to be the most reliable in our laboratory for CMV.

Double stranded RNA (dsRNA) was extracted from infected kava leaves or from indicator plants using the CF-11 column chromatography procedure of Morris and Dodds (1979). The dsRNA pellet from 5 g of tissue was resuspended in 100 μL TE buffer, pH 7.4
(Sambrook et al. 1989), and 20 μL of this preparation was used for electrophoretic analysis. Nucleic acids were separated on 1.2% agarose gels cast and run in TBE buffer, pH 8.3 (Sambrook et al. 1989). Gels were run at 100 volts for 1.5 h, stained with ethidium bromide, and photographed using UV transillumination and Polaroid film. DNA molecular weight markers for agarose electrophoresis were pGEM-3 digested with restriction endonucleases Hinfl, Rsal and SinI (Promega). dsRNA molecular weight markers were dsRNAs from rice dwarf virus kindly supplied by Dr T. Natsuaki.

Results and Discussion

The first sample received was from Fiji and was used for negative staining, IEM, dsRNA analysis and mechanical inoculation. Many samples had symptoms suggestive of virus infection and some resembled those we had occasionally seen associated with CMV in other hosts. Some yellow blotching similar to AMV infection was also present. We chose the two antisera for our IEM tests for these reasons. The only virus-like particles seen in negatively stained preparations were isometric, and in low concentration. There were no virions on the grid coated with anti-AMV but the grids coated with anti-CMV had isometric particles of 29–30 nm diameter characteristic of CMV (Figure 1). Particles resembling CMV were also detected in leaves of Nicotiana tabacum L. and N. glutinosa L. inoculated from the infected kava leaves. The leaves of the indicator species showed a strong dark-green/light-green mosaic. Extracts from the kava sample produced a dsRNA pattern which was typical of a cucumovirus (Dodds et al. 1984) and which was also present in preparations from the indicator hosts N. tabacum and N. glutinosa inoculated from infected kava (Figure 2). There were no dsRNA bands indicative of viruses of any other group in any samples.

Leaf samples selected from different cultivars at different times of the year were analysed for CMV, mostly by IEM and some by ELISA. The results for samples tested by both methods were correlated. In the ELISA tests healthy tobacco samples had a mean absorbance of 0.095; kava classified as negative, 0.118; and positive kava, 1.95. Of 55 samples, mostly from diseased plants in Tonga, 26 (44%) were positive for CMV. The most frequently examined cultivar was Kava Leka Kula (39 samples) and of these 24 (61%) were infected with CMV.

Symptoms on the positive specimens varied greatly from sample to sample and included: yellow/brown...