

Mosaic disease of vanilla (*Vanilla planifolia* Andrews)-the first report from India

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Received 27 February 2002; Revised 21 November 2003; Accepted 20 June 2003

Abstract

Recent disease surveys revealed the occurrence of a mosaic disease in vanilla. The infected plants showed mosaic symptoms, distortion of leaves, leaf curling etc. which are typical symptoms of viral infection. Negative staining of the leaf sap with potassium phosphotungstic acid, uranyl acetate and ammonium molybdate revealed the presence of flexuous rod shaped viral particles. This is the first report of occurrence of viral disease in vanilla from India.

Key words: mosaic disease, vanilla, *Vanilla planifolia*

In India large-scale cultivation of vanilla (*Vanilla planifolia* Andrews) was started only recently. The crop is mainly cultivated in several parts of southern states viz. Kerala, Karnataka and Tamil Nadu. It is one of the highly export oriented spice crops and the source for natural vanillin. Until recently, not many diseases have been noticed in this crop. But with the intensive cultivation of the crop, a number of diseases are reported in vanilla gardens. A few fungal diseases such as bean and vine rot caused by *Phytophthora* sp., *Fusarium* sp. and *Colletotrichum* sp. have been reported. A recent survey conducted in several vanilla plantations in Kerala and Tamil Nadu showed the presence of a new disease viz. mosaic or mottling characterized by leaf distortion, chlorotic streaks and leaf curling. Preliminary observations showed that the disease is not caused by fungi or bacteria, but is more similar to a viral disease. There-

fore, a detailed survey was carried out and steps for identification of the disease were taken using electron microscopy.

Field surveys for vanilla diseases were conducted during the years 2000 and 2001. In all the plantations surveyed, the vines were carefully examined, the symptoms were recorded and samples were collected for further studies. The infected plants were brought to Indian Cardamom Research Institute, Myladumpara and maintained in pot culture under greenhouse conditions. Observations on these vines were continued and the appearance of symptoms in newly emerging flushes was studied. The occurrence of mosaic disease, its severity and spread were studied in selected vanilla gardens. Representative plants with clear mosaic symptoms were taken to Central Plantation Crops Research Institute (CPCRI), Regional Station,

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Kayangulam and maintained under insect proof conditions for further studies.

Leaves showing mosaic or mottling symptoms were used for electron microscopic studies. Quick leaf-dip preparations provide the quickest method of detecting many filamentous and rod shaped viruses (Waller 2002). Electron dense stains such as 2% potassium phosphotungstic acid (KPTA), 5% of ammonium molybdate (AM) and uranyl acetate (UA) (2%) were used for negative staining. The extracted sap using different methods were placed on carbon-coated 200 mesh copper grid, dried and examined under electron microscope.

Seeds of *Nicotiana tabacum*, *Chenopodium amaranticolor*, *Vigna sinensis*, *Vinca rosea*, *Gomphrena globosa* and cluster beans were collected from Indian Institute of Horticultural Research, Bangalore and sown in sterile soil at CPCRI, Kayamkulam for transmission studies. The germinated seedlings were inoculated with sap extracted with PBS buffer from diseased plants using carborandum powder (Lucas 1967).

Among the 35 plantations surveyed, incidence of mosaic disease was observed in 9 plantations. The percentage of infection varied from 0.13-5.0% in different areas surveyed (Table 1). The common symptoms observed were mosaic and mottling of leaves (Fig. 1).

In the leaf crushing method no viral particles could be observed under EM. This is possibly due to the improper maceration of tissues and also due to the formation of con-

glomerate trapping the viral particles and hindering its visualization and identification. However in leaf bits homogenized with glass homogenizer, flexuous rod shaped viral particles could be observed in crude sap and in the centrifuged supernatant. Samples homogenized in phosphate buffer and stained with uranyl acetate showed viral particles. It

was found that staining with uranyl acetate and ammonium molybdate were comparatively better than staining with potassium phosphotungstic acid. In the preparations where viral particles were observed, the number of particles was very much less indicating low concentration of the virus. The length of the particle is approximately 650-700nm.

The presence of flexuous rod shaped particles



Fig. 1. Leaves of *Vanilla planifolia* showing mosaic symptoms

Table 1. Incidence of vanilla mosaic disease in different localities

Area/District	No. of vines	No. of vines showing mosaic	% infection
Mundakkayam/Idukki	1000	2	0.20
Churuli/Idukki	400	5	1.25
Alpara/Idukki	400	2	0.50
Chelachuvadu/Idukki	200	10	5.00
Kanchiode/Nagercoil	800	1	0.13
Alancholai/Nagercoil	800	2	0.25
Thomayarapuram/Nagercoil	400	2	0.50
Perumparai/Dindigul	100	3	3.00
Pollachi/Coimbatore	1500	15	1.00



Fig. 2. Viral particles as observed under an EM

in the preparations indicates that the disease is caused by a virus, the symptoms are almost similar to vanilla mosaic disease reported in *V. tahitensis*, *V. pompona* and *V. fragrans* from French Polynesia (Wisler *et al.* 1987; Zettler & Wisler 1990).

Transmission studies using sap of the infected plants to healthy tobacco plants (*Nicotiana tabacum*) showed mottling and crinkling symptoms within 5 days of inoculation in tobacco while *C. amaranticolor*, *V. sinensis*, *V. rosea*, *G. globosa* and cluster beans did not show any symptoms.

Besides French Polynesia, viral infections in vanilla have been reported from several other countries such as Tonga, Fiji, Cook Islands, Niue, Vanuatu etc. (Pearson *et al.* 1991; 1993). Cymbidium mosaic and Odontoglossum ring spot virus in wild and cultivated vanilla plants were reported from

Tahiti, Tahaa, Raiatea and Moorea (Wisler *et al.* 1987b). Symptoms of vanilla necrosis poty virus in *V. fragrans* reported from Fiji, Tonga and Vanuatu (Pearson & Pone 1990) were entirely different from the presently identified viral symptoms.

The flexuous rod shaped particles observed in this study indicate that the virus might belong to poty virus group. However, further confirmation by serological techniques is required to identify the specific group to which the virus belongs. This is the first report of virus disease in vanilla from this country.

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