steam-sterilized soil in greenhouse pots. Approximately 100 ml of a concentrated aqueous suspension of sclerotia from twenty-day-old petri dish cultures were added to each pot. In each crop, ten pots with five plants per pot were maintained. Plants that received water without sclerotia served as control. At flowering stage, the underground stems of the plants were injured with a sterile needle. Seven days after making the stem injury, the roots of all the plants were examined under a dissecting microscope and the infection diagnosed by the presence of distinct sclerotia. Laboratory isolation from these diseased roots confirmed the visual evaluation. Except *T. aestivum*, *C. cajan* and *D. lablab*, all the other hosts tested showed the presence of sclerotia with typical root rot symptoms. Though this pathogen has been reported on more than a hundred plant species from many parts of the world, there has been no report of this pathogen causing dry root rot disease on *C. papaya*.

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DETECTION OF INDIAN CASSAVA MOSAIC VIRUS BY ELISA

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MOSAIC disease is a major constraint in the cultivation of cassava, causing considerable yield loss[1]. It is caused by a geminivirus known as Indian cassava mosaic virus (ICMV)[2]. A similar disease in Africa is caused by African cassava mosaic virus (ACMV). ACMV and ICMV have been shown to differ in their reaction to monoclonal antibodies produced against ACMV[3]. As cassava is propagated vegetatively the disease spreads mainly due to inadvertent use of diseased stems. The damage caused by the disease can be checked to a large extent if the farmers are provided with certified healthy planting material. Main pre-requisite for such an effective screening is a reliable diagnostic method. With this aim double anti-body sandwich enzyme linked immunosorbent assay (DAS–ELISA) was tried to detect ICMV.

Antiserum to ICMV was obtained by immunizing a rabbit with ICMV purified from infected *Nicotiana benthamiana* following the method of Sequiera and Harrison[4]. DAS–ELISA tests were done as outlined by Clark and Adams[5]. Purified IgG from homologous antiserum to ICMV was used both as coating and conjugate antibody at 10^{-3} dilution. For conjugation, alkaline phosphatase (Sigma VII. P. 5521) was used in the presence of 0.05% glutaraldehyde at a concentration of 5000 units of enzyme/mg of IgG. Leaf samples were extracted with 0.01 M Tris-EDTA buffer with 0.1% Tween 20 and 2% polyvinyl pyrrolodine (MW 44,000), and two dilutions of each sample (10^{-1}, 10^{-2}) were used. For IgG coating, 200 μl of diluted preparation was put in each well of Dynatech microtitre plates and incubated at room temperature for 4 h. Plates were washed with washing buffer (PBS with 0.02% Tween 20). Immediately after washing, plates were coated with 200 μl of test samples per well. For each dilution two replicates were maintained and incubated overnight at 4°C. The plates were again washed with washing buffer before coating them with 200 μl/well conjugated antibody and incubated at room temperature for 4 h. After another round of washing 300 μl of *p*-nitrophenyl phosphate in diethanolamine buffer at pH 9.8 was added in each well and absorption read at 405 nm in a Dynatech microplate Reader-II after 1 h and overnight incubation.

Young and fully opened leaves from naturally infected plants of cassava cultivars, Kalikalan, H-226 and H-1687, showing severe symptoms were tested. In order to check whether the symptom-free plants are virus-free or not, leaves of meristem derived apparently healthy plants in the field were also assayed. Meristem derived glasshouse grown healthy cassava plants and uninoculated *N. benthamiana* were used as negative control. An absorption value

(A_{405}) twice that of the negative control were considered as positive.

ICMV could readily be detected in leaves from diseased plants of all the three cultivars showing typical symptoms, with A_{405} values ranging from 1.2 to 1.5 (table 1). No significant difference in the concentration of the virus was noticed amongst such plants of the three cultivars tested.

ICMV was also detected (table 1) in some of the apparently healthy plants. Low A_{405} values of these plants indicate smaller amount of the virus in such plants. This is expected since at higher concentration of the virus, the plants would have developed typical symptoms of the disease. A_{405} values after overnight incubation gave greater confidence in the detection of infection in apparently healthy plants as compared to values after 1 h of incubation. It is obvious particularly for cvs Kalikalan and H-1687, where similar values of positive and negative results were obtained at 1 h of incubation. These could clearly be differentiated after overnight incubation (table 1). Detection of ICMV in such symptom-free plants indicates fresh infection of the plants which were yet to develop symptoms. All meristem derived plants maintained in an insect-free glass house were not positive indicating their virus-free nature.

The apparently healthy plants in which ICMV was detected must have been freshly infected as the concentration of virus adjudged by the A_{405} values was much less in comparison with plants which had developed clear disease symptoms in all the three cultivars. Frequency of such plants was maximum in cv Kalikalan which is most susceptible of the three cultivars tested. Detection of ICMV in such symptomless plants indicates the seriousness of the problem as the farmers collect their planting material from apparently healthy plants for new plantings. Due to symptomless infection, farmer's collection of planting material will also include those from apparently healthy plants which would provide a source of inoculum in the new plantings. This may explain unexpected incidence of the disease in some of the farmers fields where apparently healthy planting materials were used. It is particularly observed in areas where vector activity is very high[1].

At present, cassava mosaic disease occurs in all parts of the country wherever cassava is grown. There is no earlier record of occurrence of the disease in places like Assam, Maharashtra and Orissa. But now it occurs. It is possible that the spread of the disease might have been due to inadvertent distribution of infected planting material collected from apparently healthy plants. This indicates the urgent need for a certification programme for healthy planting material of cassava. ELISA tests can effectively be used for certification programmes which will certainly reduce the enormous losses caused by the disease at present. It assumes special significance in the present context of introduction of cassava as industrial crop even in non-traditional areas where the disease is not prevalent.

Table 1 *Detection of ICMV in cassava by DAS–ELISA*

Cassava cultivar	Sample	Number of samples tested	Reaction	A_{405} average values after incubation	
				1 h	Overnight
Kalikalan	H	3	− 3	0	0.02
	AH	18	+ 7	0.012	0.21
			−11	0.01	0.02
	D	18	+18	1.28	1.45
H-226	H	3	− 3	0	0.02
	AH	15	+ 2	0 05	0.91
			−13	0.002	0.018
	D	13	+13	1.21	1.45
H-1687	H	3	− 3	0	0.03
	AH	16	+ 3	0.10	0 42
			−13	0.02	0.03
	D	18	+18	1.27	1.42

H, samples collected from meristem derived plants grown in a glasshouse; AH, apparently healthy plants in the field showing no symptoms; D, plants in the field showing typical mosaic symptoms.

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