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Original Research Article

Pre and Post evaluation of Direct Antigen Coating - Enzyme-Linked Immuno Sorbent Assay (DAC-ELISA) for the Detection of BBrMV and CMV in Micro-propagation of Banana

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A B S T R A C T

Plantains (*Musa* spp.)cv. Kovvur Bontha are one of the largest fruit crops produced mostly by the developing countries in the tropical and subtropical regions. Major propagule in banana cultivation is its vegetative suckers and the availability of uniform and disease-free suckers is the main problem faced by the farmers. The use of tissue culture planting material solved this problem to a greater extent by giving uniform growth and production. But there is no assurance for disease free material. Direct Antigen Coating Enzyme Linked Immuno Sorbent Assay (DAC-ELISA) was serological method to detect the presence of viral proteins. The main principle of DAC-ELISA is the detection of presence of viral protein through interaction between antigen from the coat protein with the rabbit anti-bodies. The outcome of the experiment shows that after eradication of the viral infected material at early stage will help in the production of viral free planting material. DAC-ELISA was the cheapest and easiest method to detect the virus so that we can get the good and disease-free planting material.

Keywords

DAC-ELISA, banana, Kovvur Bontha, BBrMV, and CMV

Introduction

Banana is one of the major fruit crops in India. It is also known as Adam's apple, the apple of paradise, and the poor man's apple. Banana is scientifically known as *Musa paradisiaca* L. belongs to the genus Musa of the family Musaceae of the order zingiberales. The modern edible banana is the cross between the two species *Musa accuminata* (AA) *x Musa balbisiana* (BB) which are desert type and cooking type respectively. Genome group in which A is dominant leads to the development of desert banana whereas genome group in which B is dominant leads to the development of dessert banana.

The origins of the banana are convoluted and complex. Whilst there is archaeological evidence of banana cultivation in New Guinea dating as far back as 8000 BC, other banana domestication projects have been found in Southeast Asia in the jungles of Malaysia, Indonesia, and the Philippines. Generally, it is agreed that bananas originated in Southeast Asia and the South Pacific around 8000 to 5000 BC. Bananas are believed to have been the world's first cultivated fruit. From Southeast Asia, the fruit was brought west by Arab conquerors and then carried to the New World by explorers and missionaries. Although bananas started to be traded internationally by the end of the fourteenth century, it wasn't until 1834 that the fruit was mass produced. From the late 1880s mass production of bananas exploded across the globe (Nelson *et al.,* 2006).

It plays a critical role in the income security of many tropical and sub-tropical countries. In India, the crop is mainly grown in Tamil Nadu, Karnataka, Andhra Pradesh, Maharastra, Gujarat, Kerala, Assam, West Bengal, Bihar, Madhya Pradesh, Odisha, Chhattisgarh, and Uttar Pradesh with a total area of 8.8 lakh ha producing 30 lakh tonnes. In

Kerala, banana is grown in almost all districts with an area of 1.85 lakh ha and production of 11.6 lakh tones (NHB, 2018).

Banana fruit is botanically known as berry mostly parthenocarpic in nature (triploid cultivars) with rudimentary seeds. Desert type of banana contains less starch compared to cooking type. Cooking banana contains Fat (0.22 g), Protein (2 g), Carbohydrates (58 g), Fiber (3 g), Potassium (663 mg), Vitamin C (23 mg), Vitamin A (63 ug), Vitamin B-6 (0.29 mg), and Magnesium (57 mg).

Banana is majorly propagated through suckers especially sword suckers as banana is a parthenocarpy and containing rudimentary seeds. Suckers propagated plants have a bad reputation for transferring many harmful fungal, bacterial, and viral diseases from generation to generation and land to land (Kamat and Patel, 1951). CMV is the most widely spread virus, infecting banana crops which belongs to the genus Cucumo virus and it is transmitted by aphids. Management of these vectors are difficult (Teycheney *et al.,* 2005). Viral diseases like banana bract mosaic virus (BBrMV) and cucumber mosaic virus (CMV) are the two major diseases that decrease the yield significantly and cause maximum economic losses to the farmers (Joshi snd Joshi, 1976).

Micropropagation is one of the major and effective measures to control the transfer of disease from generation to generation and land to land. It is one of the major means to avoid the transfer of viral diseases also.

Direct Antigen Coating - Enzyme-Linked Immuno Sorbent Assay (DAC-ELISA) is one of the effective serological ways to identify the presence of the virus in the suckers at earlier stages (Khan *et al.,* 2012). This helps in avoiding the transfer of dormant viral genetic material in the multiplication cycle of micropropagation (Capoor and Verma, 1968. And Kiranmai *et al.,* 1996).

The objective of this experiment is to confirm that early detection of the viruses like BBrMV and CMV in the mother helps to avoid the transfer of viral genetic material through different multiplication cycles of micropropagation and helps develop virus-free and quality planting material.

Materials and Methods

DAC-ELISA for detecting CMV was carried out in polystyrene plates (Cp-Star) using protocol described by Clark ad Adams, 1977. Healthy 120 plants were selected from the mother block for tissue culture and tagged for the assessment of viral diseases. 5g of sample was collected from the newly emerged third leaf from each plant was collected.

Direct Antigen Coating Enzyme Linked Immuno Sorbent Assay (DAC-ELISA) was serological method to detect the presence of viral proteins. The main principle of DAC-ELISA is the detection of presence of viral protein through interaction between antigen from the coat protein with the rabbit anti-bodies.

In this method 5g tissue sample was grinded with Coating Buffer (CB). After that, centrifuging at 5000rpm for 12 mins. After that 200µl of sample supernatant was loaded in 'Nuno-Maxisorp' plate with buffer and incubated at 37^{o}C for 1 hour. Later ELISA plate was washed three times with washing buffer (PBS-T buffer). Then 200µl of blocking buffer solution was added to the plate and incubated at 37^{o}C for 45 min - 1 hour. Again, the plates were washed with washing buffer for three times. Then these ELISA plates were added with primary antibodies at a dilution of 1:1000 vv^{-1} and incubated for overnight at 4^{0}C.

Next day, the plate was washed with PBS-T buffer and 200µl of secondary antibody at a dilution of 1:1000 vv^{-1} was added and incubated for 2 hours. In the next step, plates were added 100 µl of PNPP substrate into each well and cover the plates and incubate in dark at room temperature. The plates were read in ELISA plate reader. In the case of positive reaction, the colorless substrate will turn to light yellow and then to deep-yellow color. Light yellow color indicates week positive and deep yellow indicates a strong positive. Samples showing A_{405} values twice or more than healthy sample reading are considered as virus positive. The results were expressed as percent virus present among the population.

This procedure was conducted before and after the micropropagation to confirms that there are no traces of transfer of viral protein from mother plants to daughter propagules (Fig. 1).

Results and Discussion

Several workers worked on DAC-ELISA for the banana in different genomic groups and concluded that it is an effective serological method for the detection of virus. In the present experiment, 120 plant samples were subjected to the detection of two major banana viruses namely banana bract mosaic virus (BBrMV) and cucumber mosaic virus (CMV) with DACELISA.

Among 120 samples, sample number 16 (1.105), 17 (1.127), 18(0.846), 19 (1.603), 22 (1.087), 32 (1.253), 39 (1.713), 44 (1.419), 49 (1.422), 53 (1.479), 71 (1.429), 80 (1.276), 81 (1.310), 83 (1.030), 87 (1.586), 91 (1.608), 97 (1.246), 100 (1.249), and 111 (1.450) are tested positive for banana bract mosaic virus (BBrMV). It comprises of 15.6% of the total selected mother plants. These plants are discarded before going for micro propagation. Remaining explant materials are free from the virus and good for micropropagation (Table 1).

The mother plants selected for micropropagation is also subjected to undergo DAC-ELISA test for detection of cucumber mosaic virus (CMV). Sample number 8 (1.556), 19 (1.580), 22 (1.110), 30

(1.139), 43 (1.422), 49 (1.581), 60 (1.031), 61 (1.875), 72 (1.521), 73 (1.536), 86 (1691), 90 (1.780), 92 (1.518), 106 (1.678), 107 (1.504), and 109 (1.297) are detected positive for CMV which resulted in 13.3% of total mother plants selected for the explant as presented in Table 1. 3.36% of the total plants are infected with both type of the viruses which are plant sample number 19 (1.603 and 1.580), 22 (1.087 and 1.110), and 49 (1.422 and 1.581).

Post micropropagation evaluation for the detection of virus for BBrMV and CMV had shown the total negative (100%) for the presence of dormant viral protein when the 120 random propagules are selected from micropropagated plant population and subjected for DAC-ELISA test.

The data presented in the Table 2 represents the absence of viruses in the plant propagules. Which confirms the using of DAC-ELISA was the effect measure for the detection and selection of virus free material for micropropagation and planting in farmers' field.

Results of this experiment stated that early detection of the virus in banana helps to eradicate the infected material from the propagules and production of disease-free planting material for the further field transplant. DAC-ELISA is the effective means to detect the presence of viral protein. Similar findings were reported by earlier workers (Kiranmai *et al.,* 1996; Dheepa and Paranjothi, 2010, Ali *et al.,* 2012, Khan *et al.,* 2011, Selvarajan *et al.,* 2011, Kouadio *et al.,* 2014, Khan *et al.,* 2012, Lepcha *et al.,* 2017)

Singh *et al.,* (1996) reported that the concentration of BBrMV was found to be higher in infected bract as compared to healthy leaf sheath which is in unison with the result of the present study. Selvarajan, (2000) reported higher intense reaction for younger leaves when compared to older leaf. Many workers successfully detected BBrMV in infected banana by ELISA (Singh *et al.,* 1996; Reddy *et al.,* 2000; Thomas *et al.,* 2001; Kiranmai *et al.,* 2005; Selvarajan and Balasubramanian, 2008). DAC- ELSIA was found to be more economical method for the detection of BBrMV (Dhanya *et al.,* 2007).

DAC-ELISA method was found to be very efficient in detecting symptom less plant. The results revealed that positive plants recorded a mean absorbance (A405 values) which recorded more than twice the mean absorbance of negative control. Results of four plants showing different infectious chlorosis symptoms from insect proof net house recorded positive with little variation in absorbance value.

In general, higher titers of virus was recorded in positive plant followed by plant showing chlorotic lesion, mosaic with extreme distortion and reduction of leaf lamina recording absorbance value (Mali and Deshpande, 1975; Mali and Rajegore, 1979, Bouhida and Lockhart, 1990, Jones, 1991, Jones and Lockhart, 1993, Vishnoi *et al.,* 2013, Lepcha *et al.,* 2017).

Careful selection of virus-free planting material, use of resistant *Musa* varieties and farm sanitation may have been the reason for this discrepancy. This study contradicted the earlier hypothesis by Gauhl *et al.,* 1997 that local *Musa* cultivars were resistant to BSV or less prone to symptom expression. CMV was associated with BBMV infections that had CMV-like symptoms and also was associated with some, but not all, of the BBMV-infected banana plants showing characteristic bract mosaic symptoms.

Table.1 Data represents the DAC-ELISA results of pre-micropropagation for the selection of virus free explants.

Plant Sample	Banana Bract Mosaic Virus (BBrMV)				Cucumber Mosaic Virus (CMV)			
	OD* for Sample	OD for Blank	OD for Positive	Result	OD for Sample	OD for Blank	OD for Positive	Result
Plant Sample 1	0.897	0.428	1.556	Negative	0.105	0.453	1.542	Negative
Plant Sample 2	0.546	0.428	1.556	Negative	0.15	0.453	1.542	Negative
Plant Sample 3	0.595	0.428	1.556	Negative	0.197	0.453	1.542	Negative
Plant Sample 4	0.393	0.428	1.556	Negative	0.117	0.453	1.542	Negative
Plant Sample 5	0.306	0.428	1.556	Negative	0.145	0.453	1.542	Negative
Plant Sample 6	0.248	0.428	1.556	Negative	0.348	0.453	1.542	Negative
Plant Sample 7	0.413	0.428	1.556	Negative	0.094	0.453	1.542	Negative
Plant Sample 8	**0.397**	**0.428**	**1.556**	**Negative**	**1.468**	**0.453**	**1.542**	**Positive**
Plant Sample 9	0.121	0.428	1.556	Negative	0.12	0.453	1.542	Negative
Plant Sample 10	0.54	0.428	1.556	Negative	0.264	0.453	1.542	Negative
Plant Sample 11	0.714	0.428	1.556	Negative	0.107	0.453	1.542	Negative
Plant Sample 12	0.314	0.428	1.556	Negative	0.304	0.453	1.542	Negative
Plant Sample 13	0.828	0.438	1.033	Negative	0.52	0.258	0.709	Negative
Plant Sample 14	0.615	0.438	1.033	Negative	0.702	0.258	0.709	Negative
Plant Sample 15	0.726	0.438	1.033	Negative	0.465	0.258	0.709	Negative
Plant Sample 16	**1.105**	**0.438**	**1.033**	**Positive**	**0.515**	**0.258**	**0.709**	**Negative**
Plant Sample 17	**1.127**	**0.438**	**1.033**	**Positive**	**0.583**	**0.258**	**0.709**	**Negative**
Plant Sample 18	**0.846**	**0.438**	**1.033**	**Positive**	**0.156**	**0.258**	**0.709**	**Negative**
Plant Sample 19	**1.603**	**0.438**	**1.033**	**Positive**	**1.58**	**0.258**	**0.709**	**Positive**
Plant Sample 20	0.716	0.438	1.033	Negative	0.42	0.258	0.709	Negative
Plant Sample 21	0.312	0.438	1.033	Negative	0.041	0.258	0.709	Negative
Plant Sample 22	**1.087**	**0.438**	**1.033**	**Positive**	**1.11**	**0.258**	**0.709**	**Positive**
Plant Sample 23	0.878	0.438	1.033	Negative	0.212	0.258	0.709	Negative
Plant Sample 24	0.871	0.438	1.033	Negative	0.557	0.258	0.709	Negative
Plant Sample 25	0.246	0.238	1.382	Negative	0.207	0.289	0.681	Negative
Plant Sample 26	0.212	0.238	1.382	Negative	0.21	0.289	0.681	Negative
Plant Sample 27	0.213	0.238	1.382	Negative	0.23	0.289	0.681	Negative
Plant Sample 28	0.249	0.238	1.382	Negative	0.765	0.289	0.681	Negative
Plant Sample 29	0.208	0.238	1.382	Negative	0.586	0.289	0.681	Negative
Plant Sample 30	**0.253**	**0.238**	**1.382**	**Negative**	**1.139**	**0.289**	**0.681**	**Positive**
Plant Sample 31	0.215	0.238	1.382	Negative	0.54	0.289	0.681	Negative
Plant Sample 32	**1.253**	**0.238**	**1.382**	**Positive**	**0.721**	**0.289**	**0.681**	**Negative**
Plant Sample 33	0.23	0.238	1.382	Negative	0.758	0.289	0.681	Negative
Plant Sample 34	0.225	0.238	1.382	Negative	0.678	0.289	0.681	Negative
Plant Sample 35	0.196	0.238	1.382	Negative	0.504	0.289	0.681	Negative
Plant Sample 36	0.178	0.238	1.382	Negative	0.712	0.289	0.681	Negative
Plant Sample 37	0.587	0.22	1.07	Negative	0.647	0.276	0.958	Negative
Plant Sample 38	0.472	0.22	1.07	Negative	0.543	0.276	0.958	Negative
Plant Sample 39	**1.713**	**0.22**	**1.07**	**Positive**	**0.817**	**0.276**	**0.958**	**Negative**
Plant Sample 40	0.44	0.22	1.07	Negative	0.548	0.276	0.958	Negative

*OD – Optical Density

Plant Sample	Banana Bract Mosaic Virus (BBrMV)				Cucumber Mosaic Virus (CMV)			
	OD* for Sample	OD for Blank	OD for Positive	Result	OD for Sample	OD for Blank	OD for Positive	Result
Plant Sample 41	0.471	0.22	1.07	Negative	0.592	0.276	0.958	Negative
Plant Sample 42	0.412	0.22	1.07	Negative	0.528	0.276	0.958	Negative
Plant Sample 43	**0.358**	**0.22**	**1.07**	**Negative**	**1.422**	**0.276**	**0.958**	**Positive**
Plant Sample 44	1.419	0.22	1.07	Positive	0.683	0.276	0.958	Negative
Plant Sample 45	0.472	0.22	1.07	Negative	0.483	0.276	0.958	Negative
Plant Sample 46	0.481	0.22	1.07	Negative	0.623	0.276	0.958	Negative
Plant Sample 47	0.442	0.22	1.07	Negative	0.502	0.276	0.958	Negative
Plant Sample 48	0.484	0.22	1.07	Negative	0.686	0.276	0.958	Negative
Plant Sample 49	**1.422**	**0.343**	**1.702**	**Positive**	**1.581**	**0.339**	**0.984**	**Positive**
Plant Sample 50	0.551	0.343	1.702	Negative	0.804	0.339	0.984	Negative
Plant Sample 51	0.482	0.343	1.702	Negative	0.857	0.339	0.984	Negative
Plant Sample 52	0.373	0.343	1.702	Negative	0.562	0.339	0.984	Negative
Plant Sample 53	**1.479**	**0.343**	**1.702**	**Positive**	**0.653**	**0.339**	**0.984**	**Negative**
Plant Sample 54	0.458	0.343	1.702	Negative	0.652	0.339	0.984	Negative
Plant Sample 55	0.504	0.343	1.702	Negative	0.748	0.339	0.984	Negative
Plant Sample 56	0.448	0.343	1.702	Negative	0.721	0.339	0.984	Negative
Plant Sample 57	0.542	0.343	1.702	Negative	0.649	0.339	0.984	Negative
Plant Sample 58	0.419	0.343	1.702	Negative	0.641	0.339	0.984	Negative
Plant Sample 59	0.396	0.343	1.702	Negative	0.567	0.339	0.984	Negative
Plant Sample 60	**0.591**	**0.343**	**1.702**	**Negative**	**1.031**	**0.339**	**0.984**	**Positive**
Plant Sample 61	**0.553**	**0.474**	**1.417**	**Negative**	**1.875**	**0.336**	**0.915**	**Positive**
Plant Sample 62	0.63	0.474	1.417	Negative	0.691	0.336	0.915	Negative
Plant Sample 63	0.516	0.474	1.417	Negative	0.347	0.336	0.915	Negative
Plant Sample 64	0.341	0.474	1.417	Negative	0.499	0.336	0.915	Negative
Plant Sample 65	0.553	0.474	1.417	Negative	0.812	0.336	0.915	Negative
Plant Sample 66	0.675	0.474	1.417	Negative	0.78	0.336	0.915	Negative
Plant Sample 67	0.395	0.474	1.417	Negative	0.603	0.336	0.915	Negative
Plant Sample 68	0.31	0.474	1.417	Negative	0.518	0.336	0.915	Negative
Plant Sample 69	0.539	0.474	1.417	Negative	0.775	0.336	0.915	Negative
Plant Sample 70	0.311	0.474	1.417	Negative	0.603	0.336	0.915	Negative
Plant Sample 71	**1.429**	**0.474**	**1.417**	**Positive**	**0.652**	**0.336**	**0.915**	**Negative**
Plant Sample 72	**0.323**	**0.474**	**1.417**	**Negative**	**1.521**	**0.336**	**0.915**	**Positive**
Plant Sample 73	**0.443**	**0.451**	**1.287**	**Negative**	**1.536**	**0.362**	**0.819**	**Positive**
Plant Sample 74	0.596	0.451	1.287	Negative	0.664	0.362	0.819	Negative
Plant Sample 75	0.561	0.451	1.287	Negative	0.819	0.362	0.819	Negative
Plant Sample 76	0.569	0.451	1.287	Negative	0.826	0.362	0.819	Negative
Plant Sample 77	0.588	0.451	1.287	Negative	0.747	0.362	0.819	Negative
Plant Sample 78	0.57	0.451	1.287	Negative	0.597	0.362	0.819	Negative
Plant Sample 79	0.515	0.451	1.287	Negative	0.654	0.362	0.819	Negative
Plant Sample 80	**1.276**	**0.451**	**1.287**	**Positive**	**0.845**	**0.362**	**0.819**	**Negative**

*OD – Optical Density

Plant Sample	Banana Bract Mosaic Virus (BBrMV)				Cucumber Mosaic Virus (CMV)			
	OD* for Sample	OD for Blank	OD for Positive	Result	OD for Sample	OD for Blank	OD for Positive	Result
Plant Sample 81	**1.31**	**0.451**	**1.287**	**Positive**	**0.905**	**0.362**	**0.819**	**Negative**
Plant Sample 82	0.854	0.451	1.287	Negative	0.714	0.362	0.819	Negative
Plant Sample 83	**1.03**	**0.451**	**1.287**	**Positive**	**0.919**	**0.362**	**0.819**	**Negative**
Plant Sample 84	0.976	0.451	1.287	Negative	0.84	0.362	0.819	Negative
Plant Sample 85	0.664	0.474	1.417	Negative	0.875	0.336	0.83	Negative
Plant Sample 86	**0.636**	**0.474**	**1.417**	**Negative**	**1.691**	**0.336**	**0.83**	**Positive**
Plant Sample 87	**1.586**	**0.474**	**1.417**	**Positive**	**0.347**	**0.336**	**0.83**	**Negative**
Plant Sample 88	0.507	0.474	1.417	Negative	0.499	0.336	0.83	Negative
Plant Sample 89	0.813	0.474	1.417	Negative	0.812	0.336	0.83	Negative
Plant Sample 90	**0.816**	**0.474**	**1.417**	**Negative**	**1.78**	**0.336**	**0.83**	**Positive**
Plant Sample 91	**1.608**	**0.474**	**1.417**	**Positive**	**0.603**	**0.336**	**0.83**	**Negative**
Plant Sample 92	**0.553**	**0.474**	**1.417**	**Negative**	**1.518**	**0.336**	**0.83**	**Positive**
Plant Sample 93	0.732	0.474	1.417	Negative	0.775	0.336	0.83	Negative
Plant Sample 94	0.55	0.474	1.417	Negative	0.603	0.336	0.83	Negative
Plant Sample 95	0.728	0.474	1.417	Negative	0.652	0.336	0.83	Negative
Plant Sample 96	0.59	0.474	1.417	Negative	0.521	0.336	0.83	Negative
Plant Sample 97	**1.246**	**0.238**	**1.382**	**Positive**	**0.207**	**0.289**	**0.681**	**Negative**
Plant Sample 98	0.212	0.238	1.382	Negative	0.21	0.289	0.681	Negative
Plant Sample 99	0.213	0.238	1.382	Negative	0.23	0.289	0.681	Negative
Plant Sample 100	**1.249**	**0.238**	**1.382**	**Positive**	**0.765**	**0.289**	**0.681**	**Negative**
Plant Sample 101	0.208	0.238	1.382	Negative	0.586	0.289	0.681	Negative
Plant Sample 102	0.253	0.238	1.382	Negative	0.139	0.289	0.681	Negative
Plant Sample 103	0.215	0.238	1.382	Negative	0.54	0.289	0.681	Negative
Plant Sample 104	0.253	0.238	1.382	Negative	0.721	0.289	0.681	Negative
Plant Sample 105	0.23	0.238	1.382	Negative	0.758	0.289	0.681	Negative
Plant Sample 106	**0.225**	**0.238**	**1.382**	**Negative**	**1.678**	**0.289**	**0.681**	**Positive**
Plant Sample 107	**0.196**	**0.238**	**1.382**	**Negative**	**1.504**	**0.289**	**0.681**	**Positive**
Plant Sample 108	0.178	0.238	1.382	Negative	0.712	0.289	0.681	Negative
Plant Sample 109	**0.516**	**0.252**	**1.434**	**Negative**	**1.297**	**0.237**	**0.66**	**Positive**
Plant Sample 110	0.528	0.252	1.434	Negative	0.331	0.237	0.66	Negative
Plant Sample 111	**1.45**	**0.252**	**1.434**	**Positive**	**0.325**	**0.237**	**0.66**	**Negative**
Plant Sample 112	0.652	0.252	1.434	Negative	0.604	0.237	0.66	Negative
Plant Sample 113	0.592	0.252	1.434	Negative	0.389	0.237	0.66	Negative
Plant Sample 114	0.517	0.252	1.434	Negative	0.315	0.237	0.66	Negative
Plant Sample 115	0.418	0.252	1.434	Negative	0.302	0.237	0.66	Negative
Plant Sample 116	0.468	0.252	1.434	Negative	0.317	0.237	0.66	Negative
Plant Sample 117	0.487	0.252	1.434	Negative	0.266	0.237	0.66	Negative
Plant Sample 118	0.455	0.252	1.434	Negative	0.279	0.237	0.66	Negative
Plant Sample 119	0.417	0.252	1.434	Negative	0.267	0.237	0.66	Negative
Plant Sample 120	0.443	0.252	1.434	Negative	0.282	0.237	0.66	Negative

*OD – Optical Density

Table.2 Data represents the DAC-ELISA results of post-micropropagation for the selection of virus free plantlets for planting.

Plant Sample	Banana Bract Mosaic Virus (BBrMV)				Cucumber Mosaic Virus (CMV)			
	OD* for Sample	OD for Blank	OD for Positive	Result	OD for Sample	OD for Blank	OD for Positive	Result
Plant Sample 1	0.445	0.234	1.364	Negative	0.311	0.224	0.659	Negative
Plant Sample 2	0.431	0.234	1.364	Negative	0.276	0.224	0.659	Negative
Plant Sample 3	0.554	0.234	1.364	Negative	0.297	0.224	0.659	Negative
Plant Sample 4	0.532	0.234	1.364	Negative	0.290	0.224	0.659	Negative
Plant Sample 5	0.463	0.234	1.364	Negative	0.306	0.224	0.659	Negative
Plant Sample 6	0.577	0.234	1.364	Negative	0.355	0.224	0.659	Negative
Plant Sample 7	0.439	0.234	1.364	Negative	0.282	0.224	0.659	Negative
Plant Sample 8	0.438	0.234	1.364	Negative	0.288	0.224	0.659	Negative
Plant Sample 9	0.502	0.234	1.364	Negative	0.303	0.224	0.659	Negative
Plant Sample 10	0.560	0.234	1.364	Negative	0.354	0.224	0.659	Negative
Plant Sample 11	0.544	0.234	1.364	Negative	0.300	0.224	0.659	Negative
Plant Sample 12	0.502	0.234	1.364	Negative	0.310	0.224	0.659	Negative
Plant Sample 13	0.582	0.257	1.984	Negative	0.369	0.254	0.79	Negative
Plant Sample 14	0.531	0.257	1.984	Negative	0.325	0.254	0.79	Negative
Plant Sample 15	0.561	0.257	1.984	Negative	0.323	0.254	0.79	Negative
Plant Sample 16	0.631	0.257	1.984	Negative	0.355	0.254	0.79	Negative
Plant Sample 17	0.649	0.257	1.984	Negative	0.415	0.254	0.79	Negative
Plant Sample 18	0.518	0.257	1.984	Negative	0.336	0.254	0.79	Negative
Plant Sample 19	0.538	0.257	1.984	Negative	0.301	0.254	0.79	Negative
Plant Sample 20	0.602	0.257	1.984	Negative	0.321	0.254	0.79	Negative
Plant Sample 21	0.754	0.257	1.984	Negative	0.455	0.254	0.79	Negative
Plant Sample 22	0.584	0.257	1.984	Negative	0.385	0.254	0.79	Negative
Plant Sample 23	0.641	0.257	1.984	Negative	0.331	0.254	0.79	Negative
Plant Sample 24	0.560	0.257	1.984	Negative	0.333	0.254	0.79	Negative
Plant Sample 25	0.240	0.252	1.434	Negative	0.209	0.237	0.66	Negative
Plant Sample 26	0.192	0.252	1.434	Negative	0.193	0.237	0.66	Negative
Plant Sample 27	0.561	0.252	1.434	Negative	0.323	0.237	0.66	Negative
Plant Sample 28	0.631	0.252	1.434	Negative	0.355	0.237	0.66	Negative
Plant Sample 29	0.649	0.252	1.434	Negative	0.415	0.237	0.66	Negative
Plant Sample 30	0.518	0.252	1.434	Negative	0.336	0.237	0.66	Negative
Plant Sample 31	0.538	0.252	1.434	Negative	0.301	0.237	0.66	Negative
Plant Sample 32	0.584	0.252	1.434	Negative	0.385	0.237	0.66	Negative
Plant Sample 33	0.463	0.252	1.434	Negative	0.306	0.237	0.66	Negative
Plant Sample 34	0.577	0.252	1.434	Negative	0.355	0.237	0.66	Negative

*OD – Optical Density

Plant Sample	Banana Bract Mosaic Virus (BBrMV)				Cucumber Mosaic Virus (CMV)			
	OD* for Sample	OD for Blank	OD for Positive	Result	OD for Sample	OD for Blank	OD for Positive	Result
Plant Sample 35	0.439	0.252	1.434	Negative	0.282	0.237	0.66	Negative
Plant Sample 36	0.438	0.252	1.434	Negative	0.288	0.237	0.66	Negative
Plant Sample 37	0.582	0.257	1.984	Negative	0.369	0.254	0.79	Negative
Plant Sample 38	0.531	0.257	1.984	Negative	0.325	0.254	0.79	Negative
Plant Sample 39	0.561	0.257	1.984	Negative	0.323	0.254	0.79	Negative
Plant Sample 40	0.631	0.257	1.984	Negative	0.355	0.254	0.79	Negative
Plant Sample 41	0.649	0.257	1.984	Negative	0.415	0.254	0.79	Negative
Plant Sample 42	0.518	0.257	1.984	Negative	0.336	0.254	0.79	Negative
Plant Sample 43	0.538	0.257	1.984	Negative	0.301	0.254	0.79	Negative
Plant Sample 44	0.602	0.257	1.984	Negative	0.321	0.254	0.79	Negative
Plant Sample 45	0.754	0.257	1.984	Negative	0.455	0.254	0.79	Negative
Plant Sample 46	0.584	0.257	1.984	Negative	0.385	0.254	0.79	Negative
Plant Sample 47	0.641	0.257	1.984	Negative	0.331	0.254	0.79	Negative
Plant Sample 48	0.56	0.257	1.984	Negative	0.333	0.254	0.79	Negative
Plant Sample 49	0.246	0.238	1.382	Negative	0.207	0.289	0.681	Negative
Plant Sample 50	0.212	0.238	1.382	Negative	0.21	0.289	0.681	Negative
Plant Sample 51	0.213	0.238	1.382	Negative	0.23	0.289	0.681	Negative
Plant Sample 52	0.249	0.238	1.382	Negative	0.765	0.289	0.681	Negative
Plant Sample 53	0.208	0.238	1.382	Negative	0.586	0.289	0.681	Negative
Plant Sample 54	0.253	0.238	1.382	Negative	0.139	0.289	0.681	Negative
Plant Sample 55	0.215	0.238	1.382	Negative	0.54	0.289	0.681	Negative
Plant Sample 56	0.253	0.238	1.382	Negative	0.721	0.289	0.681	Negative
Plant Sample 57	0.23	0.238	1.382	Negative	0.758	0.289	0.681	Negative
Plant Sample 58	0.225	0.238	1.382	Negative	0.678	0.289	0.681	Negative
Plant Sample 59	0.196	0.238	1.382	Negative	0.504	0.289	0.681	Negative
Plant Sample 60	0.178	0.238	1.382	Negative	0.712	0.289	0.681	Negative
Plant Sample 61	0.418	0.257	1.984	Negative	0.302	0.254	0.797	Negative
Plant Sample 62	0.468	0.257	1.984	Negative	0.317	0.254	0.797	Negative
Plant Sample 63	0.487	0.257	1.984	Negative	0.266	0.254	0.797	Negative
Plant Sample 64	0.455	0.257	1.984	Negative	0.279	0.254	0.797	Negative
Plant Sample 65	0.417	0.257	1.984	Negative	0.267	0.254	0.797	Negative
Plant Sample 66	0.443	0.257	1.984	Negative	0.282	0.254	0.797	Negative
Plant Sample 67	0.546	0.257	1.984	Negative	0.15	0.254	0.797	Negative
Plant Sample 68	0.595	0.257	1.984	Negative	0.197	0.254	0.797	Negative
Plant Sample 69	0.393	0.257	1.984	Negative	0.117	0.254	0.797	Negative
Plant Sample 70	0.306	0.257	1.984	Negative	0.145	0.254	0.797	Negative
Plant Sample 71	0.248	0.257	1.984	Negative	0.348	0.254	0.797	Negative

*OD – Optical Density

Plant Sample	Banana Bract Mosaic Virus (BBrMV)				Cucumber Mosaic Virus (CMV)			
	OD* for Sample	OD for Blank	OD for Positive	Result	OD for Sample	OD for Blank	OD for Positive	Result
Plant Sample 72	0.413	0.257	1.984	Negative	0.094	0.254	0.797	Negative
Plant Sample 73	0.397	0.234	1.364	Negative	0.168	0.224	0.656	Negative
Plant Sample 74	0.121	0.234	1.364	Negative	0.12	0.224	0.656	Negative
Plant Sample 75	0.54	0.234	1.364	Negative	0.264	0.224	0.656	Negative
Plant Sample 76	0.714	0.234	1.364	Negative	0.107	0.224	0.656	Negative
Plant Sample 77	0.314	0.234	1.364	Negative	0.304	0.224	0.656	Negative
Plant Sample 78	0.44	0.234	1.364	Negative	0.548	0.224	0.656	Negative
Plant Sample 79	0.471	0.234	1.364	Negative	0.592	0.224	0.656	Negative
Plant Sample 80	0.412	0.234	1.364	Negative	0.228	0.224	0.656	Negative
Plant Sample 81	0.358	0.234	1.364	Negative	0.222	0.224	0.656	Negative
Plant Sample 82	0.419	0.234	1.364	Negative	0.283	0.224	0.656	Negative
Plant Sample 83	0.472	0.234	1.364	Negative	0.483	0.224	0.656	Negative
Plant Sample 84	0.481	0.234	1.364	Negative	0.223	0.224	0.656	Negative
Plant Sample 85	0.442	0.257	1.984	Negative	0.502	0.254	0.797	Negative
Plant Sample 86	0.484	0.257	1.984	Negative	0.286	0.254	0.797	Negative
Plant Sample 87	0.561	0.257	1.984	Negative	0.323	0.254	0.797	Negative
Plant Sample 88	0.631	0.257	1.984	Negative	0.355	0.254	0.797	Negative
Plant Sample 89	0.649	0.257	1.984	Negative	0.415	0.254	0.797	Negative
Plant Sample 90	0.518	0.257	1.984	Negative	0.336	0.254	0.797	Negative
Plant Sample 91	0.538	0.257	1.984	Negative	0.301	0.254	0.797	Negative
Plant Sample 92	0.602	0.257	1.984	Negative	0.321	0.254	0.797	Negative
Plant Sample 93	0.754	0.257	1.984	Negative	0.455	0.254	0.797	Negative
Plant Sample 94	0.584	0.257	1.984	Negative	0.385	0.254	0.797	Negative
Plant Sample 95	0.641	0.257	1.984	Negative	0.331	0.254	0.797	Negative
Plant Sample 96	0.56	0.257	1.984	Negative	0.333	0.254	0.797	Negative
Plant Sample 97	0.24	0.252	1.434	Negative	0.209	0.237	0.66	Negative
Plant Sample 98	0.192	0.252	1.434	Negative	0.193	0.237	0.66	Negative
Plant Sample 99	0.561	0.252	1.434	Negative	0.323	0.237	0.66	Negative
Plant Sample 100	0.631	0.252	1.434	Negative	0.355	0.237	0.66	Negative
Plant Sample 101	0.649	0.252	1.434	Negative	0.415	0.237	0.66	Negative
Plant Sample 102	0.518	0.252	1.434	Negative	0.336	0.237	0.66	Negative
Plant Sample 103	0.538	0.252	1.434	Negative	0.301	0.237	0.66	Negative
Plant Sample 104	0.584	0.252	1.434	Negative	0.385	0.237	0.66	Negative
Plant Sample 105	0.463	0.252	1.434	Negative	0.306	0.237	0.66	Negative
Plant Sample 106	0.577	0.252	1.434	Negative	0.355	0.237	0.66	Negative

Plant Sample 107	0.439	0.252	1.434	Negative	0.282	0.237	0.66	Negative
Plant Sample 108	0.438	0.252	1.434	Negative	0.288	0.237	0.66	Negative
Plant Sample 109	0.582	0.252	1.434	Negative	0.369	0.237	0.66	Negative
Plant Sample 110	0.531	0.252	1.434	Negative	0.325	0.237	0.66	Negative
Plant Sample 11	0.246	0.238	1.382	Negative	0.302	0.254	0.797	Negative
Plant Sample 112	0.212	0.238	1.382	Negative	0.317	0.254	0.797	Negative
Plant Sample 113	0.213	0.238	1.382	Negative	0.266	0.254	0.797	Negative
Plant Sample 114	0.249	0.238	1.382	Negative	0.279	0.254	0.797	Negative
Plant Sample 11	0.208	0.238	1.382	Negative	0.267	0.254	0.797	Negative
Plant Sample 116	0.253	0.238	1.382	Negative	0.282	0.254	0.797	Negative
Plant Sample 117	0.215	0.238	1.382	Negative	0.150	0.254	0.797	Negative
Plant Sample 118	0.253	0.238	1.382	Negative	0.197	0.254	0.797	Negative
Plant Sample 119	0.230	0.238	1.382	Negative	0.117	0.254	0.797	Negative
Plant Sample 120	0.225	0.238	1.382	Negative	0.145	0.254	0.797	Negative

*OD – Optical Density

Fig.1

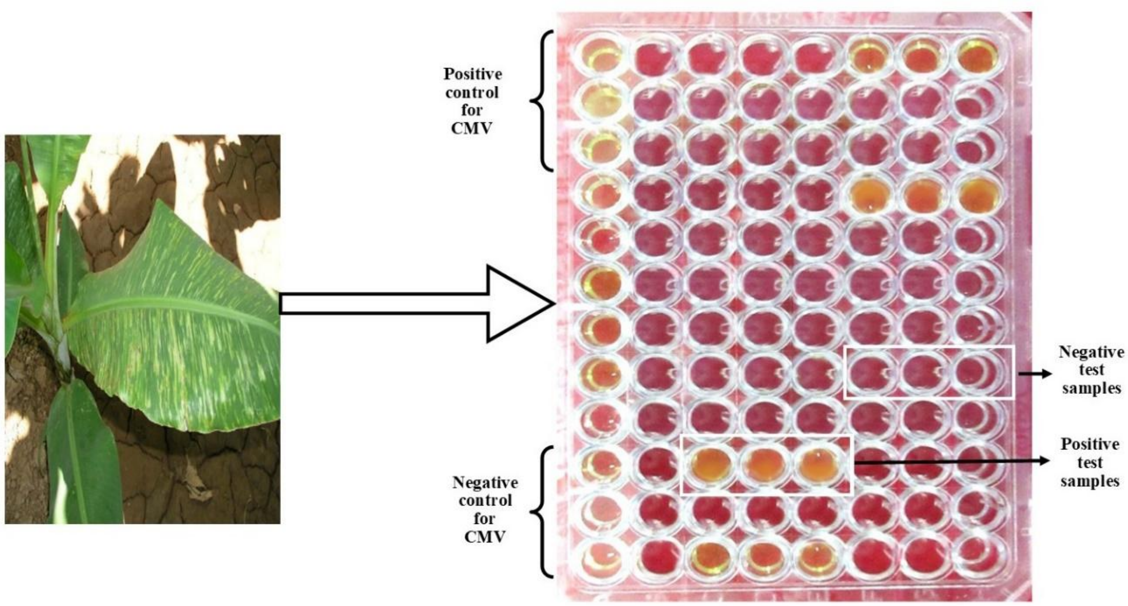

Plate 4.2: ELISA plate showing results of banana mother plant (cv. Kovvur Bontha (ABB)) samples for Cucumber Mosaic Virus (CMV) using DAC-ELISA. Samples showing positive results (yellow wells) for CMV: **(A)** white to creamish discoloration of leaves, an initial symptom of CMV **(B)** Reddish discoloration of pseudostem, an initial symptom of CMV

It appears, therefore, that CMV is not essential for the development of the characteristic BBMV symptoms. CMV may mask the development of the characteristic symptoms of BBMV in some banana cultivars, or alternatively, the CMV-like symptoms observed in the Tiruchchirappalli region may be due to a different strain of

BBMV. CMV and banana streak badna virus can cause a wide range of symptoms in banana, depending on the banana cultivar, virus strain, and environmental conditions (Jones and Lockhart, 1993, Hu *et al.,* 1995, Singh *et al.,* 1995). Therefore, the mild mosaic symptoms on the younger leaves of BBMV-infected bananas (Munez, 1992) could be confused with the mosaic symptoms produced by CMV on infected bananas (Frison and Putter, 1989).

In this experiment we concluded that the DAC-ELISA was the effective and economical method to detect and eradicate the viruses like BBrMV and CMV from mother plants to its progenies which are propagated through micropropagation.

Compliance with ethical standards

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the author.

Informed consent

Informed consent was not applicable to this article since no information regarding individual participants was included in the study.

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