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Research Paper

Viral indexing for the mass production of disease free planting materials of banana

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Abstract

Banana and plantains (*Musa* spp.) are one of the largest food-fruit crop 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. In this study, the extent of disease incidence among the suckers collected for various parts of Kerala and Tamil Nadu states were compared through viral indexing, to assess the possibility of disease incidence in the Tissue culture planting materials. From the results it was reported that among the suckers collected from Tamil Nadu State, though there was 16.7 and 12.5 percent bacterial and fungal contamination, the suckers were free from viral infection. None of the suckers collected from Tamil Nadu state showed viral infection from any of the three major viruses, while those from Kerala, 41.2, 23.5 and 16.7 percent showed bacterial, fungal and viral contamination. From the study it is concluded that the climatic condition in which the mother plants grow has an influence in the contamination status of tissue culture. If explants need to be taken from Kerala, the mother plants should be grown in protected condition with less humidity.

Keywords: Banana, viral indexing, sucker, shoot multiplication, mosaic virus

Introduction

Banana and plantains (*Musa* spp.) are one of the largest food-fruit crop produced mostly by the developing countries in the tropical and subtropical regions. Globally, banana ranked fourth, next to rice, wheat and maize in its production. It is a major staple food crop for millions of people as well as provides income through local and international trade[1]. The major problem faced by the farmers in the banana growing areas is the shortage of uniform and disease free planting materials. In conventional cultivation, it is propagated by vegetative mode through side-suckers. Production through side suckers are in slow pace and uniform growth cannot be assured. This difficulty can be solved to a greater extent by employing the commercial tissue culture technology for mass-production of the popular banana varieties. During the recent years, the adoption of high quality, disease free planting material developed through tissue culture has gained its importance in the banana growing regions. Thus the 'banana tissue culture propagation' is now become an established and popular technology as it yields large-scale production of disease-free planting materials with comparatively uniform crop stand and early harvesting of superior fruit bunches. In addition, the year round availability of the planting materials makes it more attractive.

BBTV (Banana Bunchy Top Virus), CMV (Cucumber Mosaic Virus), BBrMV (Banana Bract Mosaic Virus) are the most important virus diseases affecting bananas. Banana bunchy top is a viral disease caused by a single-stranded DNA virus called the Banana Bunchy Top Virus (BBTV) and has spread

around the world since its first identification in 1889 at Fiji, where the infected plants are stunted and have "bunchy" leaves at the top. Dale (1987) reported that the virus is present round the year in hill area but less significant in plains of India[2]. In the tropical regions, the disease is transmitted by banana aphids, which has an alternate host as *Heliconia*, a flowering ginger (from the *Zingiberaceae* family). Disease management by controlling the spread of vectors and by using disease free planting materials are the only means as there is no resistant variety. Banana plants are infected by this virus at all ages. The disease is usually spread to newer area by planting diseased suckers at the beginning of the season, which means the season is started with a diseased crop[3]. The *Banana bract mosaic virus* (BBrMV), which belongs to the genus *Potyvirus* and family *Potyviridae,* is the causal agent of bract mosaic. *Cucumber mosaic virus* (CMV) is the typical member of the *Cucumovirus* genus in the family *Bromoviridae*. It's transmission from plant to plant is by various mode like by sap, by seeds, by aphids and by the parasitic weeds, *Cuscuta sp.* (dodder). Probably this is a virus with worldwide distribution and a very wide host range. These viral diseases are causing economical loss to the banana farmers in the country.

All the three viruses, BBTV, CMV and BBrMV spread via undetected infection in suckers and the secondary spread through the banana aphid, *Pentalonia nigronervosa* in a semi- persistent manner[4-6]. The Tissue culture nutrient media in which the plant tissue is cultivated is a good source of nutrient for microbial growth. These microbes compete adversely with plant tissue culture for nutrient. In addition, it may also results in increased culture mortality, variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting[7].

There are now a range of techniques available for the detection and identification of viruses. These include observation of symptoms, examination of tissues by electron microscope and the use of indicator plants, serology and nucleic acid hybridization. Each method has certain advantages and disadvantages. Modern serological detection techniques can be highly sensitive in detecting known viruses[8]. The National Research Centre for Banana, Tiruchirapalli has developed virus-indexing techniques to control the spread of the viruses by early detection in mother plants among the tissue-culture plants. The present investigation was done to compare the intensity of viral infections in the suckers collected from within and outside the states of Kerala.

Materials and Methods

Banana suckers collected from Kerala and Tamil Nadu states were used for *invitro* shoot initiation and these *in vitro* shoots were subjected for virus indexing using DAC ELISA. The laboratory works were carried out at Rice Research Station, Kerala Agricultural University, Vyttila. A total of 99 banana suckers were subjected for multiple shoot initiation. Among these suckers, 51 suckers were from Kerala and 48 suckers were from Tamil Nadu. At first, the extent of bacterial and fungal contaminations were assessed and those with contaminations were removed from the study. Only those suckers free from bacterial and fungal contaminations were used for multiple shooting for the production of Tissue culture banana plants. At the S4 stage when plants attain enough growth for cutting 1 g size from it, the plants were subjected for viral indexing as per standard procedure of Direct Antigen Coating Enzyme Linked Immuno Sorbent Assay (DAC ELISA). 1g tissue sample was ground with Coating Buffer (CB). After centrifuging at 5000rpm, 200µl of sample supernatant was loaded with buffer and incubated at 37^{o}c for 1 hour. Later ELISA plate was washed 3 times with wash buffer. Incubated at 37^{o}c for 1 hour / 45 min. after adding 200µl of Blocking Solution. Again plate was washed in ELISA Washer. After adding 200µl of Primary Antibody Solution, it is incubated at 4^{o}c for overnight. Next day, the plate was washed and 200µl of secondary Antibody was added which is again incubated for 2 hours. After incubating at room temperature in dark for ½ - 1 hour by adding substrate solution, it was subjected for reading in ELISA reader.

Results and Discussion

Viral diagnosis of a virus infection in plants is the easy method of detection, which was developed at the National Research Centre for Banana, Tiruchirapalli. Banana viruses known to exist in latent form without expressing any visual symptoms for long time. If mother plants used for mass multiplication are nit indexed, the virus can easily pass through to the progenies. Hence it is a need to standardize and develop serodiagnostic techniques to identify the viruses, if they are in latent form.

In tissue culture, contamination can originate from two ways, either through the explants, or through faulty handling by the workers in the laboratory. Plant surfaces are habitats for microorganisms[9]. During the developmental stages, many surface and rhizosphere-inhabiting microorganisms may enter the tissues of the plant through natural openings, wounds etc. and colonies the plant tissues. In addition, facultative and obligate pathogens may also colonies plants similarly[10].

Details of samples tested for each virus are furnished in Table 1 and 2. In this, investigation was done to compare the contamination level of suckers from Kerala and Tamil Nadu. A total of 51 suckers were collected from Kerala and 48 suckers were collected from Tamil Nadu. Bacterial and fungal contaminations can be visually expressed. The fungal and bacterial contamination score of the present study revealed that the suckers collected from Kerala have higher % of bacterial (41.2 %) and fungal (23.53 %) contamination compared to the suckers collected from Tamil Nadu (16.67% and 12.5 % respectively) (Table 2).

After eliminating those with bacterial and fungal contamination, remaining 18 samples of Kerala nad 34 samples of Tamil Nadu were carried forward to multiplication stage and viral indexing was done at S2 stage. Thus in total, 52 tissue-culture samples representing 52 mother-plant samples were tested against viruses. The number of positive samples for BBTV was zero, while there was two positive samples for BBrMV and one positive sample for CMV.

In case of viral contamination, it cannot be visually identified. Viruses are invisible and will remain inside the culture. In future it will grow as a diseased plant. Virus will only be in active form after we plant the plantlets. The phenotypic symptoms of viral infection will only be expressed after its growth. In large plantations, when the sample mother sucker collected for tissue culture is virally contaminated, the whole micro propagated plantlets from the mother sucker will also be infected and it will be a huge loss to the farmers. In case of viral contamination, none of the suckers from Tamil Nadu showed contamination but four suckers from Kerala had contamination.

After virus indexing, the presence of BBrMV and CMV were confirmed in the suckers collected from Kerala region. The presence of BBrMV was confirmed in three samples and that of CMV was confirmed in one sample. There is no viral contamination in the sample suckers collected from Tamil Nadu region, which might be due to the low humidity in the environment. But the suckers collected from Kerala showed more contamination of bacteria, fungus and virus. This might be due to the high humidity in the environment. In mass multiplication through tissue culture, a single explant will be multiplied into 1000 – 1200 plants. Hence, if single explants is infected with virus, all the resultant tissue culture seedlings will express the symptom during growth and the net result will be the complete crop loss. As per National Certification System, all the explants used for tissue culturing should be free from virus and have to be confirmed through virus indexing. The tissue culture seedlings produced even after virus indexing of explants should be rechecked in random for confirmation.

Table 1: Details of samples tested for banana viruses

No.	Sample Label	BBTV		BBrMV		CMV	
1	KB-1	2.184	Negative	0.148	Negative	0.292	Negative
2	KB-2	2.597	Negative	0.160	Negative	0.378	Negative
3	KB-3	1.963	Negative	0.145	Negative	0.319	Negative
4	KB-4	1.297	Negative	0.141	Negative	0.317	Negative
5	KB-5	2.422	Negative	0.104	Negative	0.307	Negative
6	KB-6	2.827	Negative	0.146	Negative	0.348	Negative
7	**KB-7**	**2.746**	**Negative**	**0.170**	**Negative**	**0.828**	**Positive**
8	KB-8	2.538	Negative	0.165	Negative	0.320	Negative
9	KB-9	2.678	Negative	0.162	Negative	0.320	Negative
10	KB-10	3.172	Negative	0.160	Negative	0.434	Negative
11	KB-11	1.672	Negative	0.146	Negative	0.301	Negative
12	**KB-12**	**0.814**	Negative	**0.461**	**Positive**	**0.281**	**Negative**
13	KB-13	1.642	Negative	0.158	Negative	0.314	Negative
14	**KB-14**	**2.039**	Negative	**0.417**	**Positive**	**0.306**	**Negative**
15	KB-15	0.976	Negative	0.122	Negative	0.325	Negative
16	KB-16	0.95	Negative	0.122	Negative	0.282	Negative
17	KB-17	0.692	Negative	0.116	Negative	0.266	Negative

No.	Sample Label	BBTV		BBrMV		CMV	
18	KB-18	1.152	Negative	0.139	Negative	0.364	Negative
19	TB-1	2.301	Negative	0.145	Negative	0.248	Negative
20	TB-2	2.707	Negative	0.136	Negative	0.224	Negative
21	TB-3	3.038	Negative	0.181	Negative	0.324	Negative
22	TB-4	3.276	Negative	0.138	Negative	0.276	Negative
23	TB-5	2.957	Negative	0.156	Negative	0.272	Negative
24	TB-6	1.508	Negative	0.131	Negative	0.185	Negative
25	TB-7	1.569	Negative	0.149	Negative	0.161	Negative
26	TB-8	1.364	Negative	0.122	Negative	0.162	Negative
27	TB-9	1.434	Negative	0.122	Negative	0.171	Negative
28	TB-10	1.773	Negative	0.131	Negative	0.175	Negative
29	TB-11	1.622	Negative	0.137	Negative	0.168	Negative
30	TB-12	1.65	Negative	0.127	Negative	0.172	Negative
31	TB-13	1.648	Negative	0.128	Negative	0.165	Negative
32	TB-14	1.714	Negative	0.126	Negative	0.168	Negative
33	TB-15	1.851	Negative	0.123	Negative	0.180	Negative
34	TB-16	3.178	Negative	0.169	Negative	0.347	Negative
35	TB-17	1.462	Negative	0.133	Negative	0.161	Negative
36	TB-18	2.174	Negative	0.139	Negative	0.308	Negative
37	TB-19	2.916	Negative	0.137	Negative	0.322	Negative
38	TB-20	3.316	Negative	0.156	Negative	0.345	Negative
39	TB-21	2.602	Negative	0.133	Negative	0.415	Negative
40	TB-22	2.398	Negative	0.143	Negative	0.222	Negative
41	TB-23	2.078	Negative	0.145	Negative	0.236	Negative
42	TB-24	2.466	Negative	0.148	Negative	0.277	Negative
43	TB-25	2.44	Negative	0.163	Negative	0.305	Negative
44	TB-26	1.703	Negative	0.139	Negative	0.269	Negative
45	TB-27	2.069	Negative	0.146	Negative	0.267	Negative
46	TB-28	3.056	Negative	0.182	Negative	0.308	Negative
47	TB-29	2.585	Negative	0.159	Negative	0.292	Negative
48	TB-30	2.547	Negative	0.281	Negative	0.243	Negative
49	TB-31	1.914	Negative	0.149	Negative	0.236	Negative
50	TB-32	2.407	Negative	0.163	Negative	0.258	Negative
51	TB-33	2.155	Negative	0.168	Negative	0.259	Negative
52	TB-34	1.683	Negative	0.132	Negative	0.250	Negative
Negative Values		**5.354**		**0.334**		**0.694**	

KB stands for Kerala Banana TB stands for Tamil Nadu Banana

No of sample tested	No of positives BBTV	CMV	BBrMV
Kerala samples 18	All negative	1	2
Tamil Nadu samples 34	All negative	All negative	All negative

Table 2: Bacterial, Fungal and Viral contaminants during initiation of banana culture

Sucker Type	No. initiated	Bacterial contamination		Fungal contamination		Viral contamination	
		No.	%	No.	%	No.	%
Kerala Banana	51	21	41.2 %	12	23.53 %	3	16.67 %
Tamil Nadu Banana	48	8	16.67 %	6	12.5 %	0	0 %

Among those 51 from Kerala region 21 were having bacterial contamination and 12 were having fungal contamination. From those 48 in Tamil Nadu region, eight were having bacterial and six were having fungal contamination. Of the remaining suckers 16.67 % of Suckers collected from Kerala were reported to have viral contamination, while of the suckers from Tamil Nadu recorded positive values for the three viruses tested.

Conclusion

Bananas are heavily contaminated with one or more viruses. Sensitive virus detection methods are essential for developing a virus free foundation stock for tissue culture. Tissue culture is the modern technology of micro-propagation that is applied in the advanced countries for mass production of clones of superior grade planting material for most crops. Contamination in tissue culture can originate from two sources, either through carry-over of microorganisms on the surface or in the tissues of explants, or through faulty procedures in the laboratory. Plant surfaces are habitats for microorganisms. These microbes, may enter the tissues through natural openings, wounds etc. and develop endophytic 'floras' consisting of inter-and intracellular microorganisms including viruses, viroids, prokaryotes (bacterial and bacteria-like agents) and fungi. At present, different methods are available for the detection and identification of plant viruses. Among these, serological techniques are based upon the specific recognition of viral antigens by antibodies, and are frequently preferred because of their speed, specificity, and simplicity. BBTV (Banana Bunchy Top Virus), CMV (Cucumber Mosaic Virus), BBrMV (Banana Bract Mosaic Virus) are the most important virus diseases affecting bananas. In the present investigation, a comparative study was made to check the contamination level of banana suckers collected from Kerala State and Tamil Nadu. The Kerala climate is hot humid which favours disease development whereas the Tamil Nadu climate is less humid, which is not ideal for disease development. The results of this study indicated that the chances of contamination for the explants collected from less humid area (Tamil Nadu) is low as compared with those collected from more humid area (Kerala) The contamination rate of suckers collected from Kerala, was very high (25.5% bacterial contamination, 16.7% fungal contamination and 7.8% viral contamination) whereas the contamination rate was less (15.7% bacterial contamination, 10.4% fungal contamination and no viral contamination) in case of explants taken from Tamil Nadu suckers. From the study it is concluded that the climatic condition in which the mother plants grow has an influence in the contamination status of tissue culture. If explants need to be taken from Kerala, the mother plants should be grown in protected condition with less humidity.

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