ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

DETECTION OF BANANA STREAK VIRUS BY ELISA TECHNIQUES IN BANANA LEAVES

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Received on 12-10-2013 *Accepted on 30-10-2013*

Abstract

This paper presents the details of investigations carried out on detection of banana streak virus (BSV) in banana leaves. Direct antigen coating (DAC) - ELISA and Dot-blot-ELISA techniques have been employed for the detection of BSV in infected banana leaf. Suckers were collected from banana plants infected with possible BSV and the plants were indexed for presence of viruses by DAC-ELISA. From the studies, it is observed that BSV induced interveinal chlorotic straks of varied sizes in banana. In DAC-ELISA, BSV was detected up to 10^{-2} dilutions of tissue extracts but it was detected by Dot-blot-ELISA up to 10^{-3} dilution. Out of fifteen field samples tested for BSV, only one weakly reacted. However, in Dot-blot-ELISA, none were found positive for BSV. Out of fifteen samples, none were found mixedly infected. From the overall investigation, it is noted that Dot-blot-ELISA is comparatively more sensitive for the detection of BSV in banana.

Introduction

It is very well known that banana is one of the world's most important tropical fruit crop and also a cash crop. Banana is a humid tropic plant coming up with a temperature range of 10^0c to 40^0c and average of 23^0c. Four viruses known to naturally infect banana widely in different countries are BBTV, CMV, BBrMV and BSV [1]. Certain fungal diseases of banana are shown in Table 1 and Table 2 shows the details of virus diseases of banana. *Banana streak viruses* (BSV) are mealybug-transmitted members of the plant pararetrovirus genus *Badnavirus*, infecting banana and plantain worldwide [2,3]. BSV infections cause characteristic chlorotic and necrotic leaf streak symptoms. Depending on

infecting BSV species, highly susceptible banana cultivars can develop more severe symptoms, such as pseudostem splitting and necrosis, eventually leading to the death of infected plants [4].

Table-1: Certain fungal diseases of banana.

	Disease	**Casual organism**	**Distribution**
1	Fusarium wilt (Panama)	Fusarium oxysporum Fsp. Cubense	Australia, central America, Hawaii, Asia
2	Sigatoko leaf spots a. Yellow sigatoka b. Black Sigatoka	Mycosphaerella musicola Mycosphaerella fijiensis	Java, Fiji, Srilanka, Asia & America Fiji, Asia, Pacific, Central America.
	Other Leaf spots		
3	Coradana leaf spot	Cordana musae	Wide spread occurrence in all major banana growing areas
4	Leaf speckle	Mycosphaerella musae	Australia
5	Banana freckle	Guignardia musae	Asia-Pacific area
6	Deightoniella leaf sport	Deightoniella torulosa	
7	Malayan (or) diamond leaf spot	Haplobasidion musae	Asia-Pacific area
8	Black cross disease	Phyllachora musae	Asia-Pacific area
9	Banana rust	Uromyces musae	Asia-Pacific area
	Pre -harvest fruit spots		
1	Pitting disease	Pyricularia grisea	Pacific coastal areas of central America, west
2	Brown spot	Cercospora hayi	Indias central and south America
3	Speckle	Deightoniella forulosa	World wide
4	Fruit freckle	Phyllostictina musarum	Indian, Taiwan
	Tip-rot diseases		
1	Finger rot	Botryodiplodia theobramae	Central America, west indies
2	Cigar-end rot	Trachysphaera sructigena	West Africa & Egypt
3	Sclerotinia fruit rot	Sclerotinia sclerotiorum	Asrael & Bermuda
4	Black heart	Fusarium moniliforme	Israel
	Post-harvest diseases of fruit		
1	Crown rot	Collectotrichum musea Fusarium spp	Central America and west indies
2	Anthracnose and neck	Collectotrichum musae	-

Table-2: The reported virus diseases of banana.

S. No	**Disease**	**Casual virus**
1	Bunchy top	Banana Bunchy top virus (BBTV)
2	Infectious chlorosis	Cucumber mosaic virus (CMV)
3	Bract mosaic	Banana bract masaic virus (BBrMV)
4	Streak	Banana streak virus (BSV)
5	Mosaic	Tobacco mosaic virus (TMV)
6	Die-back	Nepovirus
7	-	Potexvirus
8	Abaca mosaic	Abaca mosaic virus (AbaMV)

Although originally not considered an economically important virus, BSV has raised strong concern over the past 15 years due to an increasing record of infections among new banana and plantain breeding lines and micropropagated hybrids. Interspecific *Musa acuminata* × *Musa balbisiana* genotypes, including a number of newly created hybrids, showed a tendency to produce BSV-infected propagules from virus-free source plants propagated by tissue culture. In view of this, there is a need to detect these viruses for the selection of virus free planting material. Planting of virus free seed or other propagation material is a prime practice for effective disease control. Dot-blot-ELISA using nitrocellulose or nylon membrane as support has been used for the detection of potato viruses initially. Subsequently, this technique has been applied for the detection of several viruses in both plant tissues and insect vectors. It was reported that Dot-blot-ELISA is a relatively more sensitive and economical in using the different reagents when compared to conventional ELISA performed in plastic plates. Further, the test sample extracts can be blotted on the membrane at the field level and send them to laborites for further processing. This indicates a wide potential application of the technique for the large scale detection of viruses.

This paper presents the details of investigations carried out on banana leaf affected by banana streak virus by using DAC ELISA and DOT-BLOT-ELISA techniques.

2. Materials and Methods

Suckers collected from banana plants infected with BSV from Kadapa district of Andhra Pradesh state, India were propagated in the garden. The plants have been indexed for presence of viruses by DAC-ELISA. The direct antigen coating (DAC) form of indirect ELISA described by Hobbs et al., [5] and Mowat and Dawson [6] have been adopted to detect viruses in plants.

Preparation of reagents:

(a) Phosphate buffered saline (PBS), pH 7.4:

Nacl	= 8.0 g
$Na_2HPO_42H_2O$	=1.44 g
KH_2PO_4	= 0.2 g
KCl	= 0.2 g
Distelled water	= 1000.00 ml

(b) Phosphate buffered saline – tween – 20 (PBS-T), pH 7.4:

0.5 ml of Tween-20 was added to 1000 ml of PBS

(c) PBS – TPO:

Polyvinyl pyrrolidine (MW 40000) = 2 g

Ovalbumin = 0.2 g

PBS – T = 100.0 ml

(d) Coating buffer, pH 9.6:

Na_2CO_3 = 1.59 g

$NaHCO_3$ = 2.93 g

DIECA = 2.25 g

Distelled water to 1000 ml

(e) Diethanolamine substrate buffer, pH 9.8

Diethanolamine = 97.0 ml

Distelled water = 800.0 ml

pH adjusted to 9.8 with IN HCl (about 67 ml), made up to 1000 ml with distelled water and stored at room temperature.

(f) Alkaline phosphates (ALP) conjugate

Goat antirabbit antibodies labeled with ALP diluted (1:5000) with PBS-TPO was used

(g) Substrate para-nitrophenylphosphate (PNP) solution

Two 5 mg tablets of PNP (Sigma) were dissolved in 20 ml of substrate buffer

(h) Antiserum

RTBV Banana antisera were used at 1:500 dilution in PBS-TPO respectively

(i) Antigen Extraction Buffer

For DAC-ELISA, virus infected and healthy leaf and pseudostern tissues were extracted in carbonate buffer containing 0.01 M DIECA

Procedure:

Antigen samples prepared in carbonate buffer were added to wells of the plate and incubated for 90 min. at 37°c. The plate was washed three times with PBS-T. RTBV and BSV-Banana antisera were added to the wells. The plate was covered with a lid and incubated at 37°c for 90 min. Then the plate was washed 3 times with PBS-T with 3 min. gap between each wash. The goat antirabbit labeled with ALP diluted (1:5000) with PBS-TPO was added to wells. The plate was incubated at 37°c for 90 min. and washed with PBS-T three times as above. The enzyme substrate PNP (sigma no-104) added to wells and incubated at room temperature for 1 hour in dark for colour development. The reaction was terminated by adding 3N NaOH solution at 50 µl/well. The reactions were noted according to colour intensity. The plate was read at A_{405}nm in ELISA plate reader.

Dot-blot-ELISA

Dot-blot-ELISA has been carried out according to the method described by Banttari and Goodwin [7] and Hibi and Satio [8].

Preparation of reagents:

(a) Coating buffer, pH 9.6

Na_2CO_3 = 1.59 g

$NaHCO_3$ = 2.93 g

Dissolved in about 900 ml distelled. H_2O, adjusted pH to 9.6 and made up to 1000 ml with distelled water.

(b) Tris – buffered saline (TBS), pH 7.5

Tris (0.02 m) = 4.84 g

NaCl (0.15 m) = 58.8 g

Dissolved in 1900 ml distelled water, adjusted pH to 7.5 and made up the volume to 2000 ml with distelled water.

(c) TBS-Tween

TBS = 1000 ml

Tween-20 = 0.5 ml

(d) Blocking solution

TBS = 100 ml

Non fat dried = 5g

Milk powder

(e) Antibody buffer

TBS-T = 100 ml

Nonfat dried milk powder = 5 g

(f) HRP labeled goat antirabbit IgG

Diluted in antibody buffer (1:5000) just before use.

(g) Substrate buffer(0.5M sodium citrate, pH 5.2)

for HRP system:

Trisodium citrate = 735 mg

Dissolved in 30 ml distilled H_2O adjusted pH to 5.2 with IN HCl and made up to 50 ml with distilled H_2O

(g) Substrate solution

For HRP system

Dissolved 6 mg DAB in 9 ml substrate buffer and added 1ml of 0.3% cobaltous chloride and 10 ml of 30% H_2O_2, mixed well and used it immediately

(h) Antisera

RTBV – heterologous antiserum and CMV banana antiserum were diluted

(i) Antigens

For Dot-blot-ELISA, virus infected (BSV) and healthy leaf and pseudostem tissues were extracted in carbonate buffer containing 0.01 M DIECA, subsequent dilutions of the antigens were made in carbonate buffer.

Antigen samples with a micropipette were applied on to the nitrocellulose membrane according to labeling. The membrane was allowed for drying and then transferred to a petriplate and blocking solution added till the membrane was fully immersed. The membrane was kept constant in blocking solution for 3 hours at room temperature with intermittent shaking. The membrane was transferred from blocking solution to diluted antiserum in blocking buffere and kept at 37^{o}c for 1 hour. The antibody solution was discarded and washed the membrane thrice with TBS-T at 5 min interval. The goat antirabbit antibodies labeled with HRP were addede to the antibody buffer and placed the membrane

in it under constant shaking conditions. The conjugate solution was discarded and the membrane was sashed thrice with TBS-T at 5 min. interval. The substrate solution specific to enzyme was added and kept in shaking till sufficient colour was developed. The membrane was washed with water and then it was treated with 1.05% sodium hypochlorite solution for decreasing the back ground colour.

Leaf and pseudostem samples from suspected banana (variety Robusta) plants in commercial gardens near Buchireddypalam Mandal, Nellore District, A.P. have been collected and tested for the presence of BSV by employing DAC-ELISA and Dot-blot ELISA described above.

Results and Discussion

In sucker propagated banana plants interveinal chlorotic straks of varied sizes were noticed on fully expanded leaves (Fig. 1). The distribution of the chlorotic streaks is not uniform throughout the leaf in certain plants. As the leaves matured, the chlorotic streaks were less prominent and in certain leaves necrosis was initiated in chlorotic streaks. More symptoms of BSV can be observed on the collected leaf.

a) An infected **(b) Healthy**

Fig. 1: Typical banana leaf.

DAC-ELISA and Dot-blot-ELISA have been used for the detection of BSV in banana (variety Robusta) collected from Buchireddypalem of Nellore District. In DAC-ELISA, BSV was detected up to 10^{-2} dilutions in leaf samples up to 10^{-1} dilution in pseudostem samples of infected banana using RTBV antiserum (Fig.2 and Table 3). But in Dot-blot-ELISA, it is observed that BSV was detected in both leaf and pseudostem samples up to 10^{-3} dilution. Further, background reaction was noticed with healthy samples in 10^{-1} dilution.

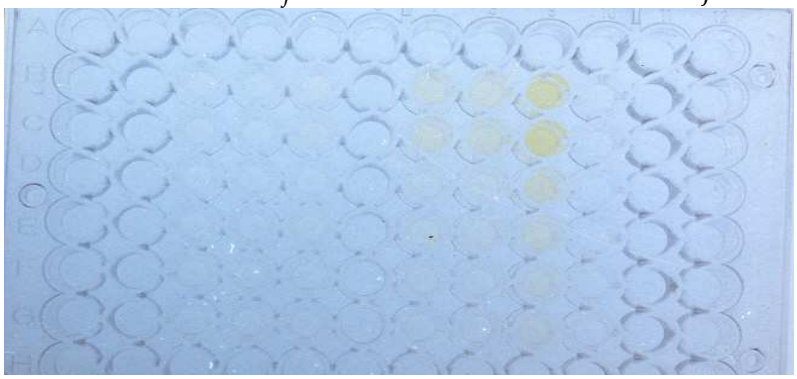

Fig. 2 Positive reactions (yellow colour) on ELISA plate showing BSV infection.

Table 3 Detection of BSV in banana samples by DAC-ELISA using heterologous RTBV polyclonal antiserum

Nature of the sample	Dilution	A_{405} reading
Healthy banana leaf extract	10^{-1}	0.10
	10^{-2}	-0.04
	10^{-3}	-0.04
Infected banana leaf extract	10^{-1}	0.32
	10^{-2}	0.21
	10^{-3}	0.02
Healthy banana Pseudostem extract	10^{-1}	0.08
	10^{-2}	-0.04
	10^{-3}	-0.04
Infected pseudostem extract	10^{-1}	0.30
	10^{-2}	0.12
	10^{-3}	0.04

Table 4 shows the details of comparison of DAC-ELISA and Dot-blot ELISA for detection of BSV in field collected banana samples. (variety: Robusta).

Sample No	**Virus**	**DAC-ELISA**	**Dot-ELISA**
1	**BSV**	0.03	-
2		0.01	-
3		0.02	-
4		0.03	-
5		0.34	-

6		0.03	-
7		0.20	-
8		0.10	-
9		0.14	-
10		0.22	-
11		0.13	-
12		0.09	-
13		0.06	-
14		0.07	-
15		0.26	-
Healthy banana leaf extract		0.21	-
BSV infected banana leaf extract		0.32	+
Healthy Rice leaf extract		0.22	-
RTBV Infected Rice Leaf extract		1.20	+

In general, it is observed from the literature that (i) Immuno-dot- ELISA on nitrocellulose membranes was used for detection of certain viruses [9]. This technique appears to have certain advantages over DAC-ELISA. Polyconal antibodies have been produced to BSV in different countries [10, 12]. The methods of detection adopted for BSV are visual infection for symptoms, immunosorbent electron microscopy, ELISA, nucleic acid hybridization, direct PCR and immuno capture PCR [10-12] . Thottappilly et al., [13] compared DAS-ELISA, TAS-ELISA, and ACP-ELISA, PAS-ELISA for the detection of BSV in banana in Nigeria. They have reported that TAS-ELISA is more sensitive than ACP-ELISA and PAS-ELISA. The concentration of virus in diseased banana is influcenced by temperature. Recently it was reported that BSV sequences may be intergated into the Musa genome (Harper et al., 1999). They have developed immuno capture-PCR to detect episomal BSV specifically

Summary and Concluding Remarks

Investigations have been carried out on infected banana leaves to detect banana streak virus. DAC-ELISA and Dot-blot-ELISA techniques have been employed for the detection of BSV in banana leaf and pseudostem tissues. The significant observations are summarized as follows:

- BSV induced interveinal chlorotic streaks of varied sizes in banana and hence difficult to identify based on visual symptoms.

- In DAC-ELISA, BSV was detected up to 10^{-2} dilutions of tissue extracts but it was detected by Dot-blot-ELISA up to 10^{-3} dilution.
- Among all the field samples tested for BSV, only one weakely reacted. However, in Dot-blot-ELISA, none were found positive for BSV.
- Among all the samples tested, none were found mixedly infected.

From the overall investigation, it is noted that Dot-blot-ELISA is relatively more sensitive for the detection of BSV in banana.

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