Detection of Banana Bunchy Top Virus in Several Aphid Species by Enzyme Linked Immunosorbant Assay (ELISA)

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ABSTRACT

Banana is very important as a fruit, vegetable and as a processed product. Banana is affected by some viral diseases including Banana Bunchy Top Virus disease, which causes damage to the yield and quality of the products. This study was carried out to test possible aphid species that transmit Banana bunchy Top Virus other than the major insect vector *Pentalonia nigronervosa*. Different types of aphid samples were collected from several host plants including banana (*Musa spp.*), chilli (*Capsicum spp.*), *Allocasia spp.*, citrus, wing beans(*Psophocarpus tetragonolobus*) and some weeds(*Gliricidia sepium* and *Miamian scandens*). Collected aphids were tested for the presence of Banana Bunchy Top Virus by indirect Enzyme linked Immunosorbant Assay using commercially available kit.

Binomial distribution analysis was done to test probability value of being positive for Banana Bunchy Top Virus due to chance of occurrence equal to 0.5. Among collected aphids, three species were identified as *Pentalonia nigronervosa, Myzus persicae, Toxoptera citricidus* according to their morphology. Thirty-three samples of aphids showed positive reaction out of forty samples based on the calculated threshold values (Sutula *et.al.*, 1986).

Binomial analysis showed being positive for any tested samples were less than 0.5. Thus, collected aphid species *of Myzus persicae, Toxoptera citricidus* might have transmitted Banana Bunchy Top Virus other than the major insect vector *Pentalonia nigronervosa*. But, further investigations are required to confirm the presence of virus in these potentional vectors.

KEYWORDS: Aphid pecies, Banana Bunchy Top Virus, ELISA

INTRODUCTION

Bananas (*Musa spp.*) not only taste terrific, they are also highly nutritious, and for this reason they are one of the most important economic food producing plants in the world (Glenn, 1987). In 2003,the extent and production of banana in Sri Lanka were 49,255 ha and 393,384 Mt, respectively. (Anon., 2004)

Banana plants are prone to a number of virus diseases such as Banana Bunchy Top Virus (BBTV), Banana Streak Virus (BSV), Cucumber Mosaic Virus (CMV) and Banana bract mosaic virus (BBrMV). (Anon., 2005) BBTV disease is widely distributed along banana growing countries. The disease was first recorded from Fiji in 1879. The disease has been recorded from many countries including Australia, Fiji, Egypt, Sri Lanka, Bonian Island and India. (Pandey, 1997). BBTV was first recorded in Sri Lanka in 1913(Gadd, 1926).

The banana plants may show the symptoms of bunchy top disease any stage of their growth. The leaves of a badly infected plant are bunched together at the apex of the plant and a rosette is formed. The further elongation of the leaf stalk is arrested and therefore, the leaves of infected plants are markedly stunted, and they do not usually grow taller than two to three feet. The irregular, nodular, dark green streaks, appear along the secondary veins on the under side of the leaf blade along the petiole or mid-rib. (Pandey, 1997)

The BBTV disease is caused by banana Bunchy Top Virus. BBTV is a Nanovirus containing multiple circular, single stranded DNA components. (Harding *et. al.*, 1991) A specific insect vector, *Pentalonia nigronervosa* coq, is transmitting the virus in persistent manner. The virus is not transmitted by mechanical inoculation. The aphids generally live round the base of pseudostem (Pandey, 1997). Like most aphids *Pentalonia nigronervosa* is a phloem feeder. This can cause plants to become deformed; the leaves become curled and shrivelled. Adult aphids are small to medium sized, shiny, redish, to dark brown or almost black. The BBTV can be detected in diseased and symptomless banana plants by ELISA with monoclonal and polyclonal antibodies (Wu and Su, 1991; 1992).

Aphis gossypii was proved to be an additional vector (Anon., 1995). There may be some other aphid species also possible to carry BBTV. Because there are a number of aphid species found near banana fields.

Therefore, the objectives of the study were to test the BBTV in major vector *Pentalonia nigronervosa* by ELISA and to test other possible aphid species that carry BBTV and further identification of different host plants for BBTV transmitting aphids.

MATERIALS AND METHODS

This study was carried out at the Plant Virus Indexing Center, Department of Agriculture, Gabadawatta, Homagama from January 2006 to July 2006.

Sample Collection

Different kinds of aphids were collected from several host plants such as banana, *Allocasia spp.*chilli, and some weed plants such as *Gliricidia*

sepium and *Miamian scandens* around Homagama area. The status of the host plants was recorded.

Identification of Aphid Species

Collected aphid species were identified according to their morphological characteristics by observing under light microscope and further identification was done according to identification guide by L.Blackman and V.F.Eastop(1980).

Testing for BBTV

Testing was done by using commercially available BBTV kit (AGDIA).

ELISA plate was coated with gamma globulins at dilution of 1:400 with carbonate buffer. The incubation period for coating was four hours at room temperature in a closed humid box. 0.1g of midrib portion of the healthy and diseased leaf pieces were used as negative and positive controls respectively. Sap was extracted from those pieces using motar and pestle. Extracted sap was diluted 1:10 in AGDIA extraction buffer (pH 7.4). Ten to twenty insects were crushed in an eppendorf tube using a toothpick and diluted by adding 300 µl of AGDIA extraction buffer. Prepared samples were centrifuge at 5000rpm for five minutes. Incubated plate was washed with PBST buffer (pH7.4). Wells were given two immediate washes and three washes at five minutes intervals. Plate was wiped well and removed air bubbles. Supernatant of the centrifuged samples was added at the rate of 100µl to a selected well. The incubation period was overnight at 4°C in a closed humid box. Washing step was done as mentioned above. Added secondary antibody (mouse anti-BBTV IgG) at the dilution of 1:400in ECI buffer. Added 100µl to a selected well. The incubation period was two hours at room temperature in a closed humid box. Washing step was done as mentioned above. Added enzyme conjugated IgG (goat anti-mouse IgG-alkaline phosphatase) with the dilution of 1:400 in ECI buffer. Added 100µl to selected well. The incubation period was one hour at room temperature in a closed humid box. Washing step was done as mentioned above. Substrate solution was prepared by adding PNP (P-nitro phenyl phosphate) to the substrate buffer (pH 9.8) Added 100µl to a selected well. The incubation period was 30 minutes at room temperature to eighteen hour at 4°C in a closed humid box. Optical density at 405nm was measured and threshold values were calculated with respect to the absorbance of healthy samples as described by Sutula *et.al.*in 1986. Above procedure was done for identified species.

Statistical Analysis

Statistical analysis was done by binomial distribution analysis to test chance of occurrence for BBTV result.

RESULTS AND DISCUSSION

Sample Collection

Aphid collected plants showed rosette like appearance and curly appearance in the leaves except *Allocasia spp.* and wing beans (Table 1). Most of the collected aphids had similar morphological characters (Table 2).

Table 1 - Aphids Collected Host Plants and Status of the Host Plants:

Host plant	Status of host plants
Banana(*Musa spp.)*	Rosette appearance
Banana(*Musa spp.)*	-
Mikania scandens	Curly
Gliricidia spp.	Curly
Chilli	Curly
Allocasia spp.	-
Wing bean	-
Citrus	Curly

(-) No any distinguish symptom

Identification of Aphid Species

Among collected aphids, three species were identified according to their morphology (Table 2) and following L.Blackman and V.F.Eastop(1980) identification key. Those aphids showed different characteristics of length, colour, antennae, dorsal abdomen, siphunculi, size and wings. Small to large size aphids were collected and also length of the body was different from each other. Among those colonies wings and wing less forms were observed. Host plants were different even in the same species. Identified species were *Pentalonia nigronervosa* with dark brown to black body, 1.2 - 1.8 mm in length, dark siphunculi and antannae longer than body (Fig.1), *Myzus persicae* with 1.8 - 2.0 mm in length, pale yellow to dark ocherous green colour body, slightly swollen on distal half of sphunculi and pale (Fig.2), *Toxoptera citricidus* with dark black body, 2.0 - 2.4 mm in length, longer, cylindrical siphunculi and pale antennae (Fig.3).

Table 2 -Morphological characters of collected aphid species:

Aphid spp.	Colour	Wings	Length (mm)	Size
Pentalonia nigronervosa	Dark brown	+/-	1.2-1.8	S M
Myzus persicae	Pale yellow	+/-	1.8-2.0	L
Toxoptera citricidus	Black	+/-	2.0-2.4	M

+/- -with wigs and wingless forms

S-Small M-Medium L-Large

Table 3 - Summary of the ELISA results for BBTV of three aphid species:

Sample No.	Aphid Species	BBTV result	Sample No.	Aphid Species	BBTV result
1	*Pentalonia nigronervosa*	+	21	*Toxoptera citricidus*	+
2	*Pentalonia nigronervosa*	+	22	*Toxoptera citricidus*	+
3	*Pentalonia nigronervosa*	-	23	*Toxoptera citricidus*	+
4	*Pentalonia nigronervosa*	-	24	*Toxoptera citricidus*	+
5	*Pentalonia nigronervosa*	-	25	*Toxoptera citricidus*	+
6	*Pentalonia nigronervosa*	-	26	*Toxoptera citricidus*	+
7	*Pentalonia nigronervosa*	+	27	*Toxoptera citricidus*	+
8	*Pentalonia nigronervosa*	+	28	*Toxoptera citricidus*	+
9	*Pentalonia nigronervosa*	+	29	*Myzus persicae*	+
10	*Pentalonia nigronervosa*	+	30	*Myzus persicae*	+
11	*Pentalonia nigronervosa*	+	31	*Myzus persicae*	+
12	*Pentalonia nigronervosa*	+	32	*Myzus persicae*	+
13	*Pentalonia nigronervosa*	+	33	*Myzus persicae*	+
14	*Pentalonia nigronervosa*	+	34	*Myzus persicae*	+
15	*Toxoptera citricidus*	-	35	*Myzus persicae*	+
16	*Toxoptera citricidus*	-	36	*Myzus persicae*	+
17	*Toxoptera citricidus*	+	37	*Myzus persicae*	+
18	*Toxoptera citricidus*	+	38	*Myzus persicae*	+
19	*Toxoptera citricidus*	+	39	*Myzus persicae*	+
20	*Toxoptera citricidus*	+	40	*Myzus persicae*	-

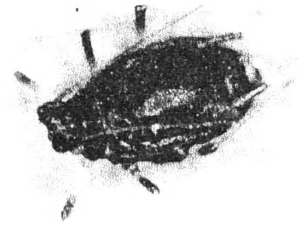

Fig. 1 - *Pentalonia nigronervosa*

Fig. 2- *Myzus persicae*

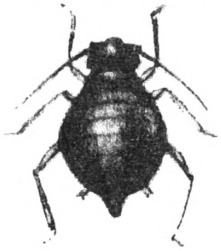

Fig. 3 - *Toxoptera citricidus*

Testing for BBTV

Forty aphid samples were tested for BBTV among three identified aphid species. Thirty-three samples showed positive reaction (Table 3) and seven samples showed negative reaction for BBTV with respect to calculated threshold values. From those collected colonies *Toxoptera citricidus* showed 30% positive for BBTV, which was the highest percentage obtained. Furthermore *Pentalonia nigronervosa* and *Myzus persicae* showed 25% and 27.5% respectively. BBTV ELISA kit specified for BBTV proteins thus, above results may be due to presence of BBTV.

Statistical Analysis

Binomial analysis was carried out to test, whether the samples are positive for BBTV is due to chance occurrence. Z calculated value was 4.11 indicating the probability value of being positive for BBTV is less than 0.5.Therefore ELISA positive results may be due to presence of BBTV. However, by insect transmission studies or by PCR, it can be further confirmed the presence of BBTV above aphid species.

CONCLUSION

The results of the study showed there might be other possible vectors for BBTV such as *Toxoptera citricidus* and *Myzus persicae* other than *Pentalonia nigronervosa*. But further investigation should be done to confirm their presence by their transmission. Weed management is essential and inter- cropping

with *Allocasia spp.* gives some risk of BBTV disease in banana fields. Some of vegetables and citrus plants might act as alternative host plants for BBTV transmitting aphids. This finding is useful in virus control programmes.

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