

**Research Article**

**AN EMERGING MALADY OF MOSAIC IN FINGER MILLET  
(*ELEUSINE CORACANA* (L.) GAERTN.) INCITED BY BADNA VIRUS**

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**ABSTRACT**

Mosaic like symptoms was observed during survey for various diseases in Finger millet in Agricultural Research Station, Vizianagaram, Andhra Pradesh. The infected leaves were collected to detect the virus from host plant tissues. Immuno-sorbent Electron Microscopy (ISEM) was done. Antisera of badnaviruses such as banana streak virus (BSV), sugarcane bacilliform virus (ScBV), Kalanchoe top-spotting virus (KTSV), Piper yellow mottle virus (PYMV), Dioscorea bacilliform virus (DBV), cacao swollen shoot virus (CSSV), Commelina yellow mottle virus (CoYMV) and rice tungro bacilliform virus (RTBV) were used. Electron microscopy of negatively stained samples from leaves of naturally infected *E. coracana* showed typical bacilliform virions measuring 100-130 x 30 nm. In decoration test all the antisera of badnaviruses viz: banana streak virus (BSV), sugarcane bacilliform virus (ScBV), Kalanchoe top-spotting virus (KTSV), Dioscorea bacilliform virus (DBV), cacao swollen shoot virus (CSSV), and Commelina yellow mottle virus (CoYMV) reacted with positively finger millet bacilliform virus. However no reaction was observed with rice tungro bacilliform virus (RTBV).

**Key Words:** Mosaic, Finger Millet, Badna viruses, Immuno-sorbent Electron Microscopy

**INTRODUCTION**

Finger millet (*Eleusine coracana* Gaertn.) known as 'ragi' in India is an important staple food for millions of people belonging to low socio-economic group. In India, it is grown in Andhra Pradesh, Maharashtra, Odisha, Tamil Nadu, Karnataka and Uttarakhand. Several reports have shown that finger millet is inexpensive and nutritionally comparable or even superior to major cereals. Regular consumption is known to reduce the risk of *Diabetes mellitus* and gastro intestinal tract disorders. It is an indispensable to Indian Agriculture as a source of grain and straw in vast dry land areas. This nutritious millet is highly nutritious and even superior to rice and wheat in certain constituents. Seeds are richest source of protein (7.3 g), crude fibre (3.6 g), mineral matter (2.7g), fat (1.3 g), carbohydrates (72g), calcium (344 mg), phosphorus (283 mg), and iron (3.9 mg) per 100 grain. The grains have high dietary fibre and helps in prevention of constipation, lowering of blood cholesterol and slow release of glucose to the blood streams during digestion. Never the less, lower incidence of cardiovascular disease, duodenal ulcer and hyperglycemia (diabetes) are reported among regular millet consumers. In India, finger millet has been grown over an area of 2.15 million hectares with an average production of 2.68 million tons (Anon, 2003).

During survey for various diseases in Finger millet in Agricultural Research Station, Vizianagaram, Andhra Pradesh, mosaic like symptoms were observed randomly in the fields. Viruses, though not common in finger millet, nevertheless have been responsible for a near total loss in the crop yield. The diseased plants have exhibited regular dark-green areas all along the leaf veins when the plants are 4-6 weeks old. Other symptoms on leaf include chlorosis and streaking. In some cases occasional yellowing symptoms were also observed. However, in the lower leaves the symptoms are of a mottle type in the form of white specks and the affected plants were stunted bearing small ear heads.

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Mosaic infected finger millet plant



Leaf showing mosaic symptoms

### MATERIALS AND METHODS

Infected leaf samples of finger millet were collected from Coastal Andhra Pradesh for the detection of viruses from host plant tissues. Immuno-sorbant Electron Microscopy (ISEM), developed by Derrick (1973) is used, a valuable technique which combines the specificity of serological test with the possibility to visualize the type of viral antigen in the electron microscope. The technique not only helps in the detection of virus particles but also facilitates virus identification. The method makes use of the trapping of antiserum-specific virus particles onto EM grids that have been coated by the specific antiserum. Samples that are serologically related to the antibodies on the coated grids get concentrated on the grids enabling the detection of virus in samples. To examine a virus in solution with the electron microscope, the sample is mounted on a grid covered with a thin film of plastic for instance formvar. These grids are copper discs with 3mm diameter, containing a number of apertures – 150 to 400 meshes/inch. The virus particles stick to the formvar, the contrast can be improved by negative staining complex. Uranyl acetate is used as negative stains which when added to a viral solution stain the background. On the screen of the EM, the virus particles are light and surrounded by a darker background.

Procedure of negative staining:

Immuno-Gold decoration technique was used. The ISEM technique has been further modified by incorporating various decoration techniques in particular gold conjugated antibodies. The sample is placed on a carbon coated grid and treated with a primary antibody. Prior to primary antibody the nonspecific binding sites are blocked by a blocking agent like BSA. The sample on the grids are reacted with a secondary antibody conjugated with 10 – 15 nm gold particles.

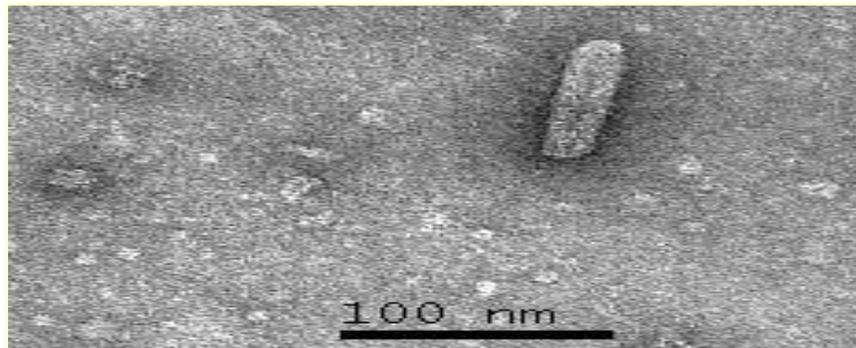
Specialized equipment, such as transmission electron microscope (TEM), carbon coating unit, refrigerator, low speed centrifuge, balances etc. Other materials like carbon coated grids, virus infected and non infected samples, crude antiserum, negative stain for instance, 2% (W/V) phosphotungstate ( $p^H$  5.5-6.0) or 2% (W/V) uranyl acetate ( $p^H$  3.7) are required to perform the test.

#### Trapping and Decoration

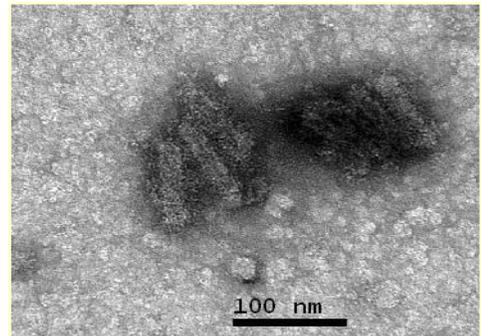
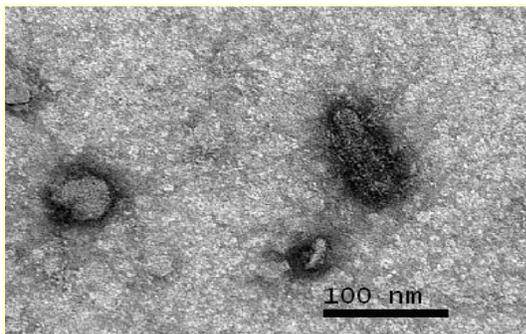
Pipette a drop of crude antisera (approximately 20 $\mu$ l) on parafilm membrane in a moist petri plate and float a grid with coated side on the drop. Incubate the petri plate for approximately 30 minutes – 1 hour at room temperature. Wash the antiserum coated grid (ACG) in phosphate buffer (0.01M,  $p^H$  7.0) for 10 min. Pipette a drop (20  $\mu$ l) of extract from diseased tissue and float the grid on it. Incubate for 30 min. to 2 hour. Wash the grid with buffer for 10 min. Pipette a drop (20  $\mu$ l) of 1:50 dilution of antiserum and place the grid on it. Incubate at 37 $^{\circ}$ C for 30 minutes to one hour. Wash the grid with water followed by 2% aqueous uranyl acetate. Drain and dry the grid before observing under electron microscope. Observe the number of virus particles trapped as compared to the leaf-dip and their decoration by the antibodies.

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Infected leaf samples of finger millet were collected from Coastal Andhra Pradesh. About 2.0 mm of infected leaf tissue were cut from symptomatic leaf and macerated in phosphate buffer (0.07 M, pH-6.5). 10 microlitre of the extract was placed on parafilm in a moist Petri plate and carbon coated copper grids (400 mesh) were placed over it. The grids were then washed with distilled water and stained with 2% freshly prepared aqueous uranyl acetate, (P<sup>H</sup>-4.5). Excess of stain was immediately removed by Whatman's filter paper. The grids were examined under JEOL, JEM-1011 transmission electron microscope (TEM) and digital images were recorded by Gatan DV 300 W CCD camera attached to EM. Thus immuno sorbent electron microscopy (ISEM) was performed. Antisera of following badnaviruses were used in ISEM tests: banana streak virus (BSV), sugarcane bacilliform virus (ScBV), Kalanchoe top-spotting virus (KTSV), Piper yellow mottle virus (PYMV), Dioscorea bacilliform virus (DBV), cacao swollen shoot virus (CSSV), and Commelina yellow mottle virus (CoYMV) and rice tungro bacilliform virus (RTBV). Antibodies of all these badna viruses were received from Prof. B.E.L. Lokhart, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108 and antibodies of RTBV was available at Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi.



**Bacilliform particle from naturally infected Finger millet in negatively stained preparations**



**Decoration of virus particles with antiserum of sugarcane bacilliform virus (SCBV). preparations.**

### RESULTS AND DISCUSSION

Electron microscopy of negatively stained samples from leaves of naturally infected *E. coracana* showed typical bacilliform virions measuring 100-130 x 30 nm. A virus concentration was very low in leaf tissue and only one particle was observed in one EM field. Approximately 4-5 particles were observed in one square of EM grid. However, in trapping with antisera of sugarcane bacilliform virus the concentration was slightly increased. In decoration test a clear antibody halo was observed around the particle. Virions were also observed in clumps showing strong reaction of decoration. In decoration test all the antisera of badnaviruses viz: banana streak virus (BSV), sugarcane bacilliform virus (ScBV), Kalanchoe top-spotting virus (KTSV), Dioscorea bacilliform virus (DBV), cacao swollen shoot virus (CSSV), and Commelina

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yellow mottle virus (CoYMV) reacted with positively finger millet bacilliform virus. However no reaction was observed with rice tungro bacilliform virus (RTBV).

Badna virus has been reported in India from banana (Anonymous, 1995), citrus (Ahlawat *et al.*, 1996), sugarcane and black pepper (Bhat *et al.*, 2003). Majority of the badna viruses are reported from vegetatively propagated plants and association of badna virus from finger millet is a new report. The particle morphology and its serological reaction in ISEM with range of badna viruses indicate that the virus belongs to genus badna virus. Similar reports were given by Patro *et al.*, (2007) for the first time in India.

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