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A SMALL RNA VIRUS ISOLATED FROM THE MAIZE STEM BORER *SESAMIA CRETICA* LED. (LEPIDOPTERA : NOCTUIDAE) IN EGYPT.

G. FEDIERE, A.A. TAHA, X. LERY, J. GIANNOTTI, J.L. ZEDDAM, A. MONSARRAT AND S. ABOL-ELA.

Entomovirology laboratory - ORSTOM - Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT

A small RNA virus was isolated from larvae of the maize stem borer *Sesamia cretica* Lederer (Lepidoptera : Noctuidae) . Some properties of this virus (Maize Stem Borer Virus : MSBV) have been studied. Electron microscopic observations of the purified suspention showed the presence of non-envelopped isometric viral particles, 30 nm in diameter. The viral genome was composed of RNA , the virus capsid contained three major proteins (VP1, VP3, VP4) with molecular weights of 60 000, 45 000 and 28 000 as well as one minor (VP2) with molecular weight of 58 000 daltons . Immunodiffusion tests showed that MSBV was serologically unrelated to certain insect Picorna-like viruses. This new virus could be provisionally arranged among the unclassified small RNA viruses of invertebrates and represents a great interst as a biological control agent .

KEY WORDS : *Sesamia cretica* , Noctuidae RNA virus , Biocontrol agent

INTRODUCTION

Corn borers are considered among the major pests in Egypt. This group of pests are not easily accessible by the methods of chemical control, hence the real insect suppression requires further control measures which have the ability to act in their target host . Among the major corn borer in Egypt, *Sesamia cretica* Lederer, 1857 (Lepidoptera : Noctuidae) is the most frequently observed, but two other lepidopterous larvae are serious pests : *Ostrinia nubilalis* Hubn. and *Chilo agamemnon* Bles. (Awadallah, 1974) . The maize stem borer S. *cretica* is a polyphagous insect on graminaceous, especially *Zea mays* , *Saccharum officiarum* and *Sorghum vulagare* (Mostafa, 1981) .

Control of this pest was limited in the use of chemical insecticides which represent at term damage for the environment. Biological control by insect viruses had to be considered . Within the genus *Sesamia* only three viruses have been described for the species *S. calamistis* Hampson (jacquemard *et al.*, 1985), i.e. Nodamuravirus, Cytoplasmic Polyhedrosis Virus and Nuclear Polyhedrosis Virus, but no viruses were recorded in *S. cretica*.

The present investigation, aims to characterize a small isometric virus isolated from dead infected larvae of *S. cretica* collocted from maize fields . Therefore, physicochemical properties, serology and electron microscopy of this virus designated Maize Stem Borer Virus (MSBV) were studied in order to provide its classification .

MATERIALS AND METHODS

LARVAE STOCKS

S. cretica larvae used in this investigation were originated from El Badrashin in the Nile valley and Kafr El Sheikh in May and June 1990. They were reared in the laboratory on semisynthetic media (Bordat, 1980).

VIRUS STRAIN

Dead infected larvae were collected from maize fields at El Badrashin in May 1990. The virus was purifid and propagated in laboratory reared larvae infected per os.

PURIFICATION OF THE VIRUS

Extract of insects, either for infectivily assays or for virus characterization, were perapred by homogenizing the infected larvae in 0.05 M Tris-Buffer (TB), pH 7.8, containing 0.5 & Sodium Dodecyl Sulfate. The extract was squeezed through cheesecloth and the emulsion was centrifuged at 8000 g for 10 minutes. The supernatant fluid was kept and the pellet was re-extract twice after re-suspending and sonificating in TB. The resulting supernatants were mixed and the virus was pelleted by centrifuging at 145000 g for 1 h 30 at 4°C. The pellet was allowed to resuspend overnight in small volum of TB.

The partially purified virus suspension was deposited on a 15 to 45 % (W/W) sucrose gradient in TB and centrifuged for 2 hours at 200000 g. The band containing virus was collected and the particales were re-purified by an additional cycle of centrifugation on sucrose gradient. The purified virus particles were then concentrated as above and stored at-30°C.

ELECTRON MICROSCOPY

Purified virus suspention was negatively stained with 2 % (W/W) uranyl acetate, pH 7.4.

DETERMINATION OF THE CHEMICAL COMPOSITION OF THE VIRION

Virus samples were tested for nucleic acid by the colorimetic method of Mejbaum (1939) using orcinol and the diphenylanine reaction (Giles and Meyers, 1965).

SPECTROPHOTOMETRIC MEASUREMENTS

U.V. absorption of purified virus was examined through wave lenghts between 320 and 220 nm . The average ratio of optical densities at 260 and 280 nm (maximum absorption of nucleic acids and proteins respectively) was mesured .

ELECTROPHORESIS OF VIRUS POLYPEPTIDES IN SDS POLYACRYANMIDE GELS

Molecular weight and number of proteins were assessed by comparing their electrorphoretic mobilities in 9% polyacrylamide gels (Weber and Osborn ,1969) with those of

standard marker proteinse : phosphorylase b (MW : 94000),bovone serum albumine (MW : 67000) , ovalbumine (MW : 43000) , carbonic anhydsrase (MW : 30000),trypsin inhibitor (MW:20100).

ANTISERA AND SEROLOGICAL TESTS

Antisera were prepared in rabbits by intraveinal injection of 1 ml antigen (500 mg / ml) and intramuscular injection twice at weekly intervals with virus preparation emulsified in Freund's complete adjuvant. Gel immunodiffiusion tests were carried out in 1 % agrose using 0.9 % NaC1 (Outcherlony, 1648). The antiserum titer was determined and the highst was selected. Reciprocal comparison of MSBV with the Picorna- like virus of *Latoia viridissima* (fediere *et al.* , 1990) and *Trunaca rufisquamata* (Fedieral *et al.*, 1991) and thier antisera were realised.

RESULTS AND DISCUSSION

Examination of purified viral suspension by electron microscope revealed large number of isometric particles, 30 nm in diameter (Fig . 1). Uv extinction spectra of the virus showed a maximum absorption at 260 nm and a minimum absorption at 240 nm, which characterize the viral nucleoproteins. The average ratio of extinction at 260 nm to that at 280 nm was 1.4 . Purified virus particales reacted negatively with diphenylalanine and positively with orcinol, which indicated the RNA nature of the viral nuclic acid. Electrophoresis of the viral proteins in 9 % polyacrylamide gels revealed three major bands with molecular weights of 60 000 (VP1) , 54 000 (VB3) and 28 000 daltons (VB4) as well as one minor band with molecular weight of 58 000 dlatons (VB2) (Fig. 2).

Immunodiffusion tests showed that MSBV virions reacted strongly with the homologous antiserum titrated by 1/128. Serological comparsion between MSBV and two Picorna- like viruses of *Latoia viridissima* and *Turnaqua rufisquamata* revealed that these viruses were serologically different. Although a large number of small RNA viruses was already isolated from insects, only a few number of these viruses were classified among three families : Picornaviridae, Nodaviridae and Teraviridae whereas the others belong to the group of unclassified small RNA viruses of invertebrates (Mattews, 1982).

Date recorded in the present investigation show that MSBV is a small, nonenvelopped, isometric virus, 30 nm in diameter . Its genome is composed of RNA and its capsid is formed of three majors and one minor structural polypeptides. From the physical and chemical properties of this RNA virus of. *S. cretica*, it is suggested to arrange it among the unclassified viruses waiting for a complete characterization. Observations throught experimentally infections realised for the production of large amount of virus (necessary for its characterization) and the natural epizootics observed in plantation, show that MSBV is highly pathogenic and give it a great interest for its use as biological control agent against *S.cretica*, this aspect will be developed in a next investigation.

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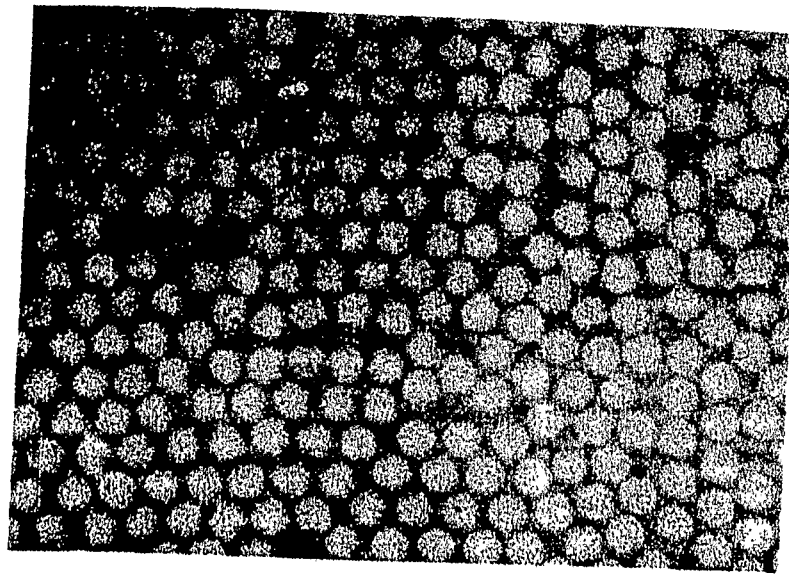

Fig. 1 : Electron micrograph of highly purified suspenion of MSBV negatively stained in 2% uranyl acetate (X 250.000).

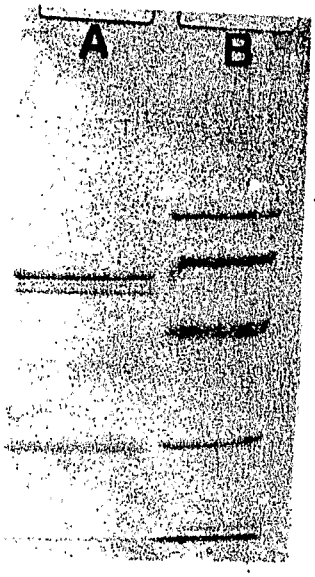

Fig. 2 : Electrophoretic analysis of polypeptides in 9% polyacrylamide-SDS gel.

Lane A : MSBV

Lane B L Protein standards : phosphorylase (MW : 94.000) , bovine serum albumin (67.000) ,ovalbumin (43.000) , carbonic anhydrase (30.000) , Trypsin inhibitor (20.100).