CHARACTERIZATION OF A PICORNA-LIKE VIRUS ISOLATED FROM THE MAIZE STEM BORER SESAMIA CRETICA LED. (NOCTUIDAE) IN EGYPT

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Summary

A small RNA virus was isolated from larvae of Sesamia Cretica (Lepidoptera Noctuidae), the most important corn borer in Egypt. Some properties of this virus (Maize Stem Borer Virus: MSBV) have been studied.

Electron microscopic observations of the purified suspension showed the presence of non-envelopped isometric viral particles, 30 nm in diameter. The virus capsid contained three major proteins (VP1, VP3, VP4) with molecular weights of 60,000, 54,000 and 28,000, as well as one minor (VP2) with molecular weight of 58,000 daltons. The viral genome was composed of one single strand RNA with molecular weight of 9,4 Kb.This viral RNA presents a terminal Poly Adenylate sequence as the members of the Picornaviridae family.

Reliable and sensitive viral diagnosis tools, based on immunoenzymatic test (ELISA), and non-radioactive nucleic probe, have been developed.

This virus is capable to cause important mortality (96 % in 8 days) by oral infection with high virus concentration, for last instard larvae. This bioassay shows that MSBV is highly pathogenic and thus presents interesting potentialities as a biocontrol agent.

Introduction

Among the major corn borer in Egypt, Sesamia cretica Lederer, 1857 (Lepidoptera: Noctuidae) is the most frequently observed. This maize stem borer is a polyphagous insect on graminaceous, especially Zea mays, Saccharum officiarum and Sorghum vulgare. Control of this pest was limited in the use of chemical insecticides, and microbiological control by insect viruses had to be considered.

Two viruses were recorded in S. cretica, i.e. Granulosis virus (ScGV) (Fédière et al., 1992 a) and small RNA virus (Fédière et al., 1991). The present investigation aims to characterize the small RNA virus designated Maize Stem Borer Virus (MSBV) in order to provide its classification.

Results

1 - Virus strain

Dead infected larvae were collected from maize fields at El Badrashin in june 1990. The virus was purified and propagated from this time in laboratory reared larvae infected per os.

2 - Electron microscopy

Examination of purified viral suspension by electron microscope revealed large number of non-envelopped isometric particles, 30 nm in diameter.

Spectrophotometric measurements

U.V. absorption of viral suspension was examined through wawelenghts between 320 and 220 nm. The average $\frac{1}{2}$ ratio of extinction at 260 nm to that at 280 nm was 1.4.

4 - Electrophoresis of the viral proteins

Molecular weigth and number of proteins were assessed by comparing their electrophoretic mobilities, in polyacrylamide gels, with those of standard marker proteins. Electrophoresis revealed three major bands with molecular weights of 60,000 (VP1), 54,000 (VP3) and 28,000 daltons (VP4), as well as one minor band with molecular weight of 58,000 daltons (VP2).

5 - Antisera and immunological tests
Antisera were prepared in rabbits. Immunoenzymatic test ELISA between MSBV and two other picorna-like viruses of Latoia viridissima (Fédière, 1983) and Turnaca rufisquamata (Fédière et al. 1992 b), revealed that these viruses were serologically different.

6 - Characterization of the viral RNA

Virus samples were tested for nucleic acid by RNAse and DNAse before electrophoresis. This test indicated the RNA nature of the genome.

The molecular weight and the number of fragments were calculated after migration in 1% agarose gel in denaturing conditions and comparison with RNAs genomes of Drosophila C virus (Jousset et al. 1977) and L. viridissima Picornavirus (Fédière et al., 1990, Zeddam et al., 1990). In these conditions the extracted RNA demonstrated to be 9.4 Kb and that only one band was present.

The retaining of the RNA by an oligo (dT) cellulose column indicated the presence of a poly(A) + tract.

The viral RNA was used as template to generate a cDNA genomic bank. Following the cloning of cDNA, plasmids were obtained containing viral inserts of different sizes. One of them, designated PSc39, contained an insert of 1.6-2.0 Kbp. The physical map of this insert revealed a Hind III-Bam HI fragment of 250 bp which was used to prepare a cold nucleic probe.

Conclusions

By severals of its properties, including a single-stranded RNA genome approximatively 9.4 Kb containing a poly(A)3' sequence this virus presents the salient features of the members of the Picornaviridae family. Both the ELISA tests and the nucleic probe provide efficients tools for epidemiological studies in field conditions.

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Microbial Control of Pests 4th European Meeting

editor:

P.H. Smits

IOBC wprs Bulletin Bulletin OILB srop

Vol. 17(3)1994