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RESTRICTION ENDONUCLEASE ANALYSIS AND DIAGNOSIS OF THE GRANULOSIS VIRUS ISOLATED FROM *SPODOPTERA LITTORALIS* BOISD. IN WEST AFRICA AND MULTIPLIED IN EGYPT.

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ABSTRACT

A Granulosis virus isolated from *Spodoptera littoralis* Boisd. (Lep. Noctuidae) in Cote d'Ivoire more than 15 years ago was multiplied in Egypt on the insect host and characterized. The size of the DNA of this Baculovirus, that we name S1GV, was estimated to be 108 Kilobases and restriction patterns have been drawn up for 15 endonucleases. Viral diagnosis methods, immunoenzymatic test. ELISA and genomic probe, have been established and tried out for epidemiological studies of the disease. The virus was compared to other GVs of *Sesamia cretica, Phthorimaea operculella, Cydia pomonella, Chilo infuscatellus* and *Cryptophlebia leucotreta* using the same technique.

Key words: Granulosis virus, Restriction endonuclease. *Spodoptera littoralis,*

INTRODUCTION

A Granulosis Virus (GV) was isolated from the Egyptian cotton leaf worm *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) more than 15 years ago in Côte d'Ivoire This species is an economically important polyphagous pest attacking cotton, vegetables, rice, maize and tobacco in many tropical and subtropical regions of Africa. The Baculovirus was purified for bioassay and for production using mass rearing of this pest on an artificial diet (Baillon, 1983). A strain of this pathogenic GV that we name S1GV

(isolate 1 from Bouake in Cote d'Ivoire) is now currently multiplied in Egypt on larvae collected from the field and reared on semi-artificial diet

The present work is considered as a part of a laboratory program concerning the inter-relationship between the different viruses isolated from *S.littoralis* and their host. The objective of the characterisation of this virus is to detect the appearence of other or local GVs infecting the same host regarding the geographical distribution of this pest, as well as the possibility of including this baculovirus in the application strategy. It is important to mention that two small RNA viruses were recentlly isolated from naturally infected larvae. and the genetic recombination among *S. littoralis* NPVs is actually under study.

In the present investigation, we report the first DNA characterization using restriction endonuclease (REN) and viral diagnosis of the *Sl*GV. Actually, work is going on to find an endemic *Sl*GV strain in Egypt which could be more adapted for the Egyptian agro-ecosystem.

MATERIALS AND METHODS

Virus isolate

The granules were obtained from natural infections of *S. littoralis* larvae collected from cotton fields. This virus was isolated in Bouake in Côte d'Ivoire and purified by Dr. Pierre Monsarrat and M.Fransois Baillon (ORSTOM). This isolate was propagated in the Egyptian *S. littoralis* larvae and the viral suspension is available at the Entomovirology Laboratory, Faculty of Agriculture, Cairo University.

Granules purification

The infected larvae were homogenized in T.S. buffer (50 mM Tris, 2 mM SDS, pH 7.8). The homogenized viral suspension was filtered and clarified, then a series of successive sedimentations on continuous sugar gradient 30/70% (p/p) using ultracentrifugation at 45 000 g for 30 minutes was conducted. The highly purified viral granules were checked by spectrophotometer DU-70 through 450 nm wavelenght. The viral suspension was stocked in Tris buffer (Tris 50 mM, pH 7.8) under - 20 ^{o}C

Electron microscopy

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

DNA extraction

The extraction of DNA from the purified GV capsules was carried out using the procedures of Fediere *et al.*, (1993). The DNA was released from purified virus particles by a treatment with 0.05 m Na2CO3, then lysed with sarkosyl and proteinase K at 37 °C within 2 hours. The exclusion of the protein from the DNA suspension was conducted through 3- phenolic extractions. The DNA was precipitated by addition of 2 volumes of absolute ethanol in presence of sodium acetate (0.3M final) for 14 hours at -20 °C. After rapid centrifugation, the pellet was washed in 70% ethanol and incubated in T.E. buffer. The concentration of DNA was finally measured according to its optic density through 260 nm wave lengh.

Restriction enzyme digestion and gel electrophoresis

Viral DNA (lug) was digested in a final volume of 20 ul, for 4 h at 37 C°, with restriction endonucleases using the conditions recommended by the suppliers (Bohreinger). Electrophoresis was carried out using 1% agarose gel in Tris-EDTA-Phosphate buffer (TEP) (90 mM Tris-Phosphate, 20 mM EDTA, pH 8.0) containing 0.5 ug/ml ethidium bromide. Electrophoresis was carried out at 50 V for 2 h. The gel was visualised and photographed on a UV transilluminator. The size of the DNA fragments was estimated by comparison with fragments of lambda DNA digested with Hind III (marker II), lambda DNA digested with both Eco RI and Hind III (marker III) and SPP1 DNA digested with Eco RI (marker VII).

Nucleic probe and hybridization

The digoxigenin-labelled S1GV DNA probe was applied according to the protocol recommended by the suppliers (Boerhinger). The same protocol was applied for the hybridization "dot blot" technique in order to determine the probe title. The Southern blot method (Southern, 1975) was applied to verify the presence of the total fragments of the genome and for detecting the sequence homology between the S1GV DNA and that of *Sesamia cretica* GV (ScGV) (Fediere *et al.*, 1993). This insect is a noctoid pest of maize in Eastern Africa and the most important borer in Egypt.

ELISA test

For detecting the Baculoviral protein, a specific rabbit serum was prepared by injecting the total protein of granules virions. The indirect method of ELISA test "Enzyme Linked Immuno Sorbent Assay" using the alkaline phosphatase was conducted according to Kelly *et al.*, (1978).

RESULTS AND DISCUSSION

The electron microscopic examination of purified viral suspension revealed the presence of ovoid-shape granules measuring 350-370 x 170-190 nm (Fig. 1) the fine observation of the granule structure indicated the viral particle envelope as well as its nucleocapsid.

The purified DNA of S1GV was digested by 15 endonucleases of current use. No restriction sites were observed when the genome was digested with Hpa I, Not I, Sma I and Sph I, while only two restriction sites were detected by Stu I giving two fragments of 7.2 and 98 Kilobases (Kb).

However, the digestion by the endonucleases Bam HI, Bgl II, Eco RI, Eco RV, Hind III, Mlu I, Pst I, Pvu II, Sal I and Xho I revealed different electrophoretic profiles composed of 11, 15, 14, 19, 18, 14, 16, 12, 12 and 11 fragments, respectively, in which the sizes are shown in Table (1).

The molecular weight of the genome was estimated by addition of the size of all fragments in each electophoretic profile. The mean of the DNA molecular weight was about 108 Kb.

Using the equivalent endonucleases for genome digestion, the electrophoretic profile of *Sl*GV was not identical with those of other GV DNAs already characterized from the following species: *Sesamia cretica,* strain of Cairo, Egypt, (Fediere *et al.*, 1993), *Chilo infuscatellus,* strain of Coimbatore, India, (Easwaramoorthy and Cory, 1990), *Phthorimaea operculella,* strain of Lima, Peru, (Vickers *et al.*, 1991), *Cryptophlebia leucotreta,* strain of Cape Verde Island, Guinea, (Jehle *et al.*, 1992) and *Cydia pomonella,* strain of Mexico, Mexico, (Crook *et al.*, 1985) (Tables 2,3,4 and 5).

A total nucleic probe labeling with Digoxigenin was prepared. The capacity of this probe for detecting the viral DNA was tested using the dot-blot technique, the deposit of 2ul was capable for detecting 5 pg of DNA. The above mentioned method was used for detecting the homology between *Sl*GV DNA and *Sc*GV DNA. The latter insect is a noctoid pest of maize in Eastern Africa and the most important borer in Egypt. No sign of recognition was found showing the specificity of the probe. The experiments of hybridization

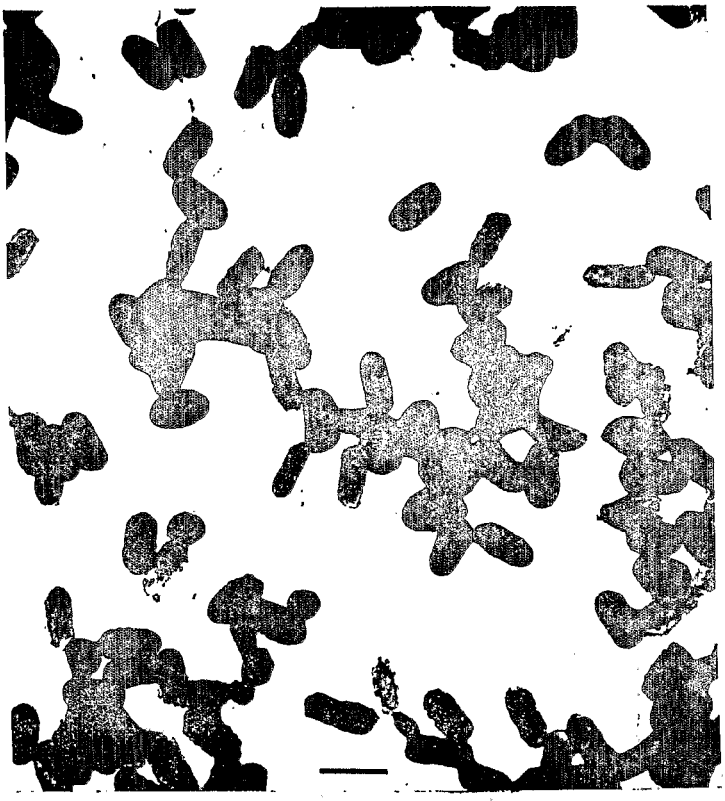

Fig. (1): Electron micrograph of purified inclusion bodies of *Spodoptera littoralis* Granulosis Virus negatively stained with uranyl acetate. Bar represents 400nm.

Table (1): Estimated size (in Kilobases) of *Spodoptera littoralis* GV DNA fragments after digestion with 10 restriction endonucleases.

	Pst I	Bgl II	BamH I	Hind III	EcoR V	Mlu I	Xho I	Pvu II	Sal I	EcoR I
A	25	20	23	22	16	21	24	22	23	21
B	21	17	20	16	12	18	17	18	20	20
C	9.9	15	16	12	9.8	14	12	15	15	19
E	7.1	14	15	8.8	8.4	12	11	13	9.4	15
D	6.9	13	13	8.2	8	7.6	6.6	7	6.9	11
G	6.7	12	12	6.3	4.8	6.2	6.5	6.5	5.9	5.9
H	5.6	10	7.6	5.7	4.6	4.9	5.7	6.3	5.9	4.4
I	4.8	8	5.4	4.9	4.1	4.4	4.9	6	5.2	4.1
J	4.3	3.8	3.8	4.6	4	4.2	4.7	5.1	3.7	2.8
K	3.8	3.5	2.6	4.4	3.5	3.5	3.8	3.3	2.9	1.7
L	3.4	2.5	1.7	3.5	3.4	2.9	2.8	3	2.8	1.6
M	3	2.1		3.3	3	2.5		2.4	2.6	1
N	2.3	1.9		3	2.7	2.1				0.9
O	1.5	1.8		2.8	2.6	1.5				0.8
P	1.3	1.4		2.1	2.4					
Q	1.1			1.6	2.2					
R				1.5	1.4					
S				1.2	1.2					
					1.1					
TOTAL	107.7	126	120.1	111.9	95.2	105	99	108	103	109.2

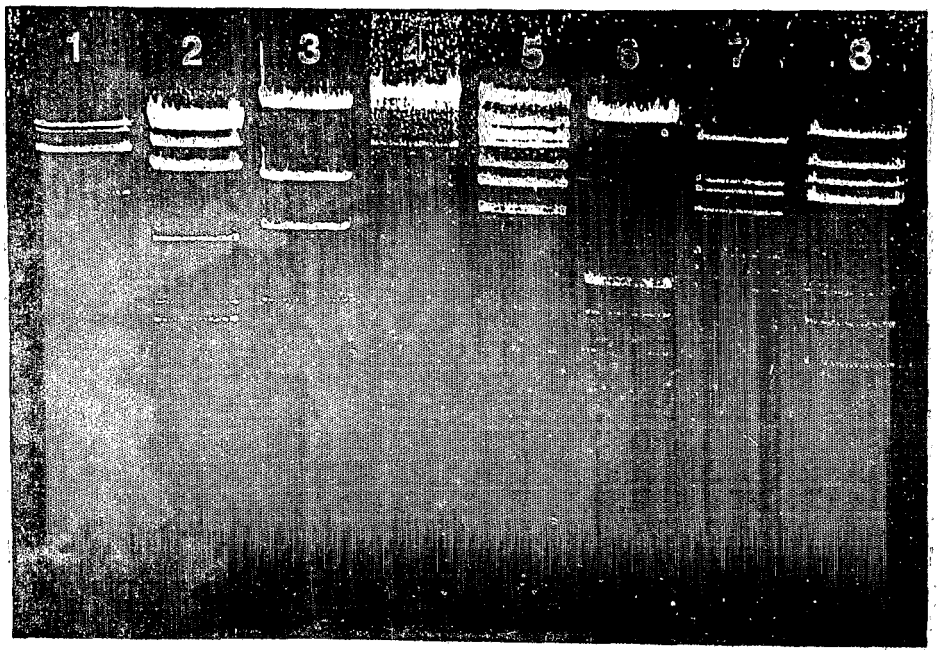

Fig. (2): Comparison of electrophoretic profiles of restricted SlGV and ScGV genomic DNA in 1% Agrose gel.

Lane 1 : Eco RI digest of ScGV DNA
Lane 2 : Eco RI digest SlGV DNA
Lane 3 : lambda DNA digested with Hind III (Marker II)
Lane 4 : Pst I digest of ScGV DNA
Lane 5 : Pst I digest of SlGV DNA
Lane 6 : lambda DNA digested with Eco RI and Hind III (Marker III)
Lane 7 : Eco RV digest of ScGV DNA
Lane 8 : Eco RV digest of SlGV DNA

Table (2): Comparison of *Spodoptera littoralis* GV, *Sesamia cretica* GV, *Phthorimaea operculella* GV, *Cydia pomonella* GV, *Chilo infuscatellus* GV and *Cryptophlebia leucotreta* GV DNA's fragments after digestion with Bam HI (in Kilobases)

	Spodoptera littoralis (Bouaké)	*Sesamia cretica* (Cairo)	*Phthorimaea operculella* (Lima)	*Cydia pomonella* (Mexico)	*Chilo infuscatellus* (Coimbatore)	*Cryptophlebia leucotreta* (Cape Verde Island)
A	23	25	25	25	19	32
B	20	21	17	25	19	16
C	16	19	11	15	16	9,85
D	15	13	11	9,5	15	9,75
E	13	10	10	9,5	8,3	7,70
F	12	7,3	9	7,0	8,3	7,50
G	7,6	5,4	8,1	6,4	7,7	6,85
H	5,4	3	8,1	5,9	6,4	6,50
I	3,8	2,3	6	5,7	3,8	6,2
J	2,6		3	5,2	2,5	5,0
K	1,7		2,85	3,9	1,0	2,6
L			0,96	3,1		2,10
M			0,88	3,1		
N			0,74	1,2		
O			0,63			
P						
Q						
R						
S						
T						
U						
V						
Total	120,1	106	114,2	125,5	106,3	112,25

Table (3): Comparison of *Spodoptera littoralis* GV, *Sesamia cretica* GV, *Phthorimaea operculella* GV, *Cydia pomonella* GV, *Chilo infuscatellus* GV and *Cryptophlebia leucotreta* GV DNA's fragments after digestion with Eco RI (in Kilobases)

	Spodoptera littoralis (Bouaké)	*Sesamia cretica* (Cairo)	*Phthorimaea operculella* (Lima)	*Cydia pomonella* (Mexico)	*Chilo infuscatellus* (Coimbatore)	*Cryptophlebia leucotreta* (Cape Verde Island)
A	21	19	19	28	16	17
B	20	18	18	22	13	12,00
C	19	15	13	17	9,3	8,50
D	15	14	13	13	7,2	8,30
E	11	7,7	12	10	7,2	8,30
F	5,9	5,1	9,4	6,4	6,7	7,60
G	4,4	4,8	7,9	5,0	6,3	7,30
H	4,1	3,7	6,6	5,0	5,1	5,5
I	2,8	2,6	3,7	4,9	4,7	5,0
J	1,7	2,4	3,2	4,8	4,3	5,0
K	1,6	2,1	2,9	3,9	4,0	4,5
L	1,0	1,6	0,6	3,1	3,8	4,0
M	0,9	1,1	0,6	1,7	3,7	4,0
N	0,8	0,9	0,1	1,1	3,5	3,70
O		0,5			3,2	2,6
P					3,2	2,2
Q					2,7	1,7
R					2,7	1,6
S					1,5	1,0
T					1,3	0,8
U					0,9	0,7
V					0,6	0,5
w						0,4
x						0,4
Total	109.2	98,5	110	125,9	112	112,83

Table (4): Comparison of *Spodoptera littoralis* GV, *Sesamia cretica* GV, *Phthorimaea operculella* GV, *Cydia pomonella* GV, *Chilo infuscatellus* GV and *Cryptophlebia leucotreta* GV DNA's fragments after digestion with Xho I (in Kilobases)

	Spodoptera littoralis (Bouake)	*Sesamia cretica* (Cairo)	*Phthorimaea operculella* (Lima)	*Cydia pomonella* (Mexico)	*Chilo infuscatellus* (Coimbatore)	*Cryptophlebia leucotreta* (Cape Verde Island)
A	24	21	28	35	22	37
B	17	19	15	24	17	24
C	12	16	12	13	11	23
D	11	13	11	8,5	11	12
E	6.6	12	10	8,1	10	12
F	6.5	7,9	9	7,2	9,50	4,6
G	5.7	5,5	6	6,7	7,00	
H	4.9	4,4	6	5,4	5,8	
I	4.7	2,9	5,6	5,1	5,6	
J	3.8	2,7	4,3	4,2	3,5	
K	2.8	2,7	4,3	3,6	3,3	
L		2,5	1,6	1,5	2,2	
M		2,4		1,3	2,1	
N		2,2		0,8	1,6	
O		1			1,6	
P		0,9			1,3	
Q		0,7				
R		0,6				
S		0,5				
T		0,4				
U		0,3				
V						
Total	99	118,8	112,40	124,4	114,60	112,60

Table (5): Comparison of *Spodoptera littoralis* GV, *Sesamia cretica* GV, *Phthorimaea operculella* GV and *Cydia pomonella* GV DNA's fragments after digestion with Hind III (in Kilobases)

	Spodoptera littoralis (Bouaké)	*Sesamia cretica* (Cairo)	*Phthorimaea operculella* (Lima)	*Cydia pomonella* (Mexico)
A	22	25	20	92
B	16	23	15	33
C	12	20	10	
D	8,8	11	9	
E	8,2	7,4	7,3	
F	6,3	7,1	7,3	
G	5,7	7	6,4	
H	4,9	6,7	5,8	
I	4,6	6,2	4,3	
J	4,4	3,4	4,0	
K	3,5	3,2	3,9	
L	3,3	2,6	3,7	
M	3,0	0,9	3,5	
N	2,8	0,7	3,3	
O	2,1	0,6	2,4	
P	1,6		1,9	
Q	1,5		1,9	
R	1,2		1,6	
S			1,6	
T			1,1	
U			0,8	
V			0,7	
Total	111,9	124,8	115,5	125

after obtaining the electrophoretic profiles of restricted *Sl*GV and *Sc*GV genomic DNA by Eco RI, Pst I and Eco V (Fig. 2), by Southern blot technique, did not permit the recognition of all the electrophoretic fragments by the probe which confirm its specificity.

An antiserum titered as 1/1200 was prepared using all dissolved proteins (the granulin and the capsid proteins). By applying the ELISA test with the alkaline phosphatase indirect method, 1 ng of the dissolved proteins was detected. An equal concentration of *Sc* GV viral proteins was less intensively visible using the same test for detection.

The partial homology between the two types of virus detected by ELISA test was due to the presence of the same sequence coding for the protein of high degree of conservation (this result was confirmed by ELISA test, while the electrophoretic profiles of the two viruses were completely different).

The two viral diagnostic tools, which were prepared and titered in the present study, represent certain importance for the study of *Spodoptera littoralis* viral epidemiology. Such a study is highly required for the determination of the viral existence among natural pest populations, as well as its persistence. These observations are needed for managing the biological control strategy.

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أستخدام التحليل الانزيمى وطرق التشخيص الفيروسية لدراسة الفيروس الجرانيولوزى لدودة ورق القطن المعزول فى غرب افريقيا وتم اكثاره فى مصر

سعيد ابو العلا – جيل فيديير – عدلى نور الدين–أميمة خميس – محسن صلاح
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ملخص

تم الاكثار المعملى للفيروس الجرانيولورى الممرض لدودة ورق القطن والمعزول منذ ١٥ عاما بكوت ديفوار ، كما تم اجراء الدراسات التشخيصية لهذا الفيروس المقترح تسميته SIGV.

وقدر حجم الحامض النووى الديزوكسى ريبوز لهذا الفيروس ب ١٠٨ الف قاعدة ، وتم تحليل الحامض النووى باستخدام ١٥ من انزيمات القطع . وبالاضافة الى الدراسات السابقة ، تم اجراء دراسات تشخيصية مثل اختبار الاليزا وذلك تمهيدا للدراسات الحقلية.

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