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CHARACTERIZATION OF TWO EGYPTIAN ISOLATES OF *SPODOPTERA LITTORALIS* (BOISDUVAL) (LEPIDOPTERA: NOCTUIDAE) GRANULOVIRUS FROM NATURAL FIELD INFESTATION IN EGYPT

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Abstract – Two natural isolates belonging to *Spodoptera littoralis* granulovirus (SlGV) were isolated from moribund cotton leafworm *S. littoralis*. They were sampled from a natural infestation of small untreated cotton fields in Gharbeia and Sharkeia governorates, Egypt. The SlGV isolates were characterized using eight restriction endonucleases. The DNA profile of both isolates were differentiated from that of the unique published isolate from the Ivory Coast Republic in Africa. The molecular weights of capsid protein were determined and compared with their homologue of the African isolate. Biotests have been carried out.

Key words – Cotton leafworm, *Spodoptera littoralis*, granulovirus, Egyptian isolate, granulovirus identification, granulovirus pathogenicity.

INTRODUCTION

The baculoviruses constitute the most practical and applicable group among insect pathogenic viruses. The major challenge that the applied entomologist has to face is to set up a control programme based on one or more baculoviruses taking into account the biological and ecological factors of the agroecosystems (BENZ 1971; ABOL-ELA *et al.* 1988, 1994; BROOKS & McLENNAM 1991; MUNOZ *et al.* 1997). The relevant research concerns the virus population analysis among naturally infected hosts, the host specificity, the identification of viral isolates and different viral clones. Such investigation is aimed to differentiate the isolates or viral clones which may express the highest virulence. A nucleopolyhedrosis virus (NPV) was isolated fourty years ago (ABUL-NASR 1956) in Egypt from the cotton leafworm *Spodoptera littoralis* (Boisduval); this virus is known by its acute pathogenicity to the young larvae. On the contrary, the *Spodoptera littoralis* granulosis virus (SlGV) expresses its effect strongly against later instars (NARAYANAN 1985). This double potentiality of viruses may be considered an advantage in the management of *S. littoralis*. Well understanding of the dosage mortality responses of an insect to virus infection is an essential requirement for the development of predictive control methods, as well as understanding of virus epizootiology. In the present study we investigated the viral genomic and protein identity of the newly found Egyptian SlGV isolates, and the quantal responses of the cotton leafworm.

MATERIALS AND METHODS

The two isolates were collected from two different locations in Egypt: in the delta from Mahala, Gharbeia governorate and from Sharkeia governorate. Granulovirus-infected larvae were homogenized and the purification of granules followed the technique of TOMPKINS (1991). The highly purified viral granules were checked by spectrophotometry, and stocked in Tris 0.01 M at – 20 °C. To obtain the virions, the purified granules were dissolved in an alkaline solution of sodium thioglycolate buffer 0.25 Mol. pH 10.5. This suspension was centrifuged, the supernatant containing virions was placed on sucrose continuous gradient from 10 to 60 % in water (w/w) and centrifuged at 18 000 rpm at 5 °C for 45 minutes using a rotor Beckman SW 28. Virions were sedimented as net band on the corresponding density gradient (SMITH & SUMMERS 1978). The virion bands were carefully collected, diluted with distilled water and pelleted. The viral DNA was extracted and separated from dissolved virion proteins by alkalin treatment and phenolic extraction. The purified DNA was then precipitated by adding ethyl alcohol and sodium acetate (CHERRY & SUMMERS 1985).

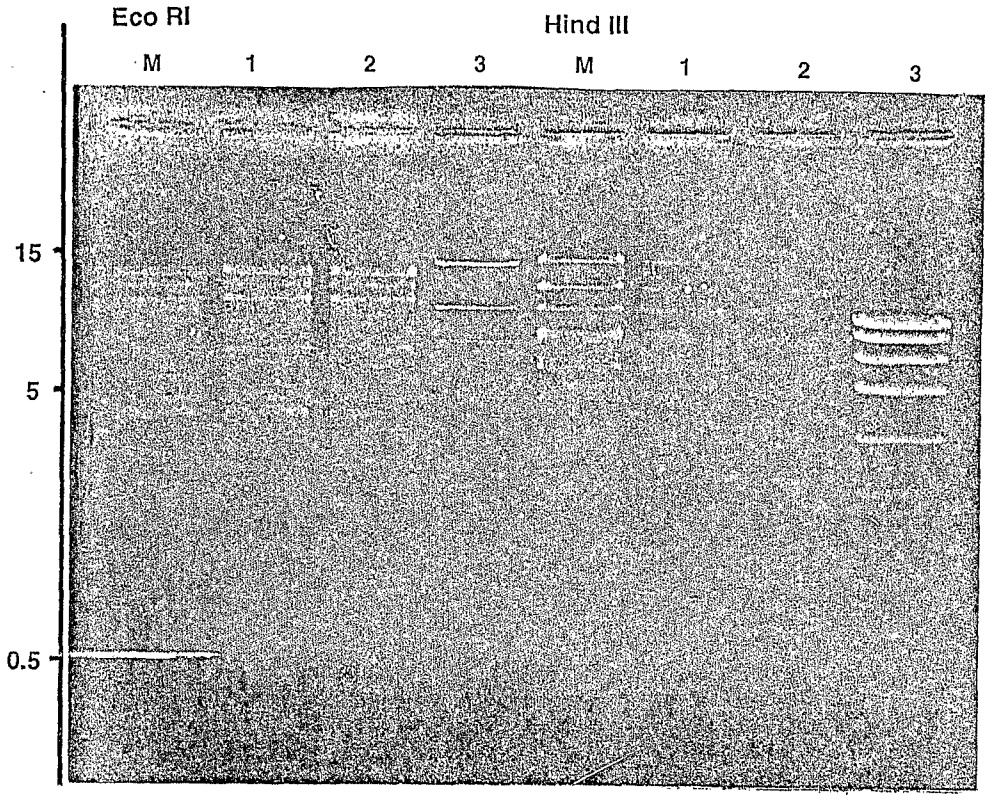

Figure 1. – Three isolates of *Spodoptera littoralis* granulovirus-DNA digested by Eco RI and Hind III. **1:** Côte d'Ivoire CD isolate; **2:** Egyptian isolate MH from Mahala; **3:** Egyptian isolate SII from Sharkia; **M:** marker.

The lethal concentration (LC) and the lethal time (LT) of SlGV were determined for the second and fourth larval instar of the cotton leafworm. A plastic plate containing 0.5-cm layer of artificial diet was divided into 84 squares of 1.5-cm side. Each plate was dosed with one of the tested concentrations, namely 119, 11.9, 1.19, 0.119, 0.0119 OD (optical density) (CHANG & TANADA 1978) in 25 µl of distilled water dispensed on the diet surface. Determination of LC and LT values was based on daily mortality records.

RESULTS AND DISCUSSION

A granulovirus (GV) was locally found from cotton leafworms and characterized in Egypt for the first time. This virus was first isolated in September 1993 from Mahala, El-Gharbeia governorate, from caterpillars showing the characteristic symptoms. Electron microscopy confirmed that the symptoms were caused by GV infection. The molecular weight of the DNA genome was estimated at about 128 kb.

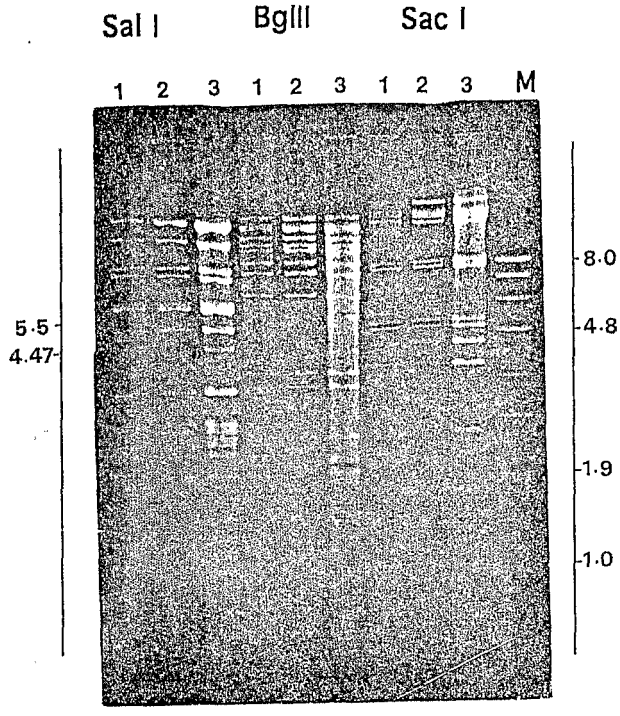

Figure 2. – Differences between the three isolates of *Spodoptera littoralis* granulovirus-DNA digested by Sal I, Bgl II, and Sac I endonucleases. **1:** MH isolate; **2:** SH isolate; **3:** CD isolate; **M:** marker.

The DNA was extracted and digested by Bgl II, Sal I, Sac I, Bam H I, Hind III, EcoR I, EcoR V, and Pst I. The viral genome profiles were compared with the isolate which is already known from the Ivory Coast Republic, Africa. The comparison demonstrated that the DNA fragments profile (genotype) of the Egyptian isolates using the first two endonuclease enzymes was not identical to the reference isolate, but closely related to (Figs 1 & 2). The

difference concerned the fragment J (approximately 5.5 kb) which appeared clearly in the African isolate but was missing in the profile of the two Egyptian isolates. The same results with the endonuclease Sal I demonstrated a missing band at the level 4.475 kb representing the fragment H in the African reference. Regarding the other used enzymes in the identification and comparison, the two isolates were almost identical and very close to the African one.

The virus was first replicated in fourth-instar larvae of *S. littoralis* collected from the same field and at the same time. Further on, the virus was replicated in insectary reared larvae. The virus was cloned *in vivo* through four passages. In addition, the SlGV was successfully replicated *in vitro* using different lepidopteran cell lines: Phop 95 from *Phthorimaea operculella* (Zeller) (LÉRY*et al.* 1997), Sl 96 from *S. littoralis,* and Sf 9 from *Spodoptera frugiperda* (J.E. Smith) (KHAMISS, unpubl. data).

The profile of the capsid proteins presented in Fig. 3 demonstrates the protein profile of the three SlGV isolates: Mahalla (MH), Sharkeia (SH) and the African one (CD) in the polyacrylamid gel. The capsid proteins were not completely identical with each other, the reference isolate and the SH Egyptian isolate are relatively close. There was one missing fragment at the level 34 kDa, and a supplementary fragment estimated at 86.667 kDa which was not present neither in the reference type nor in the SII isolate.

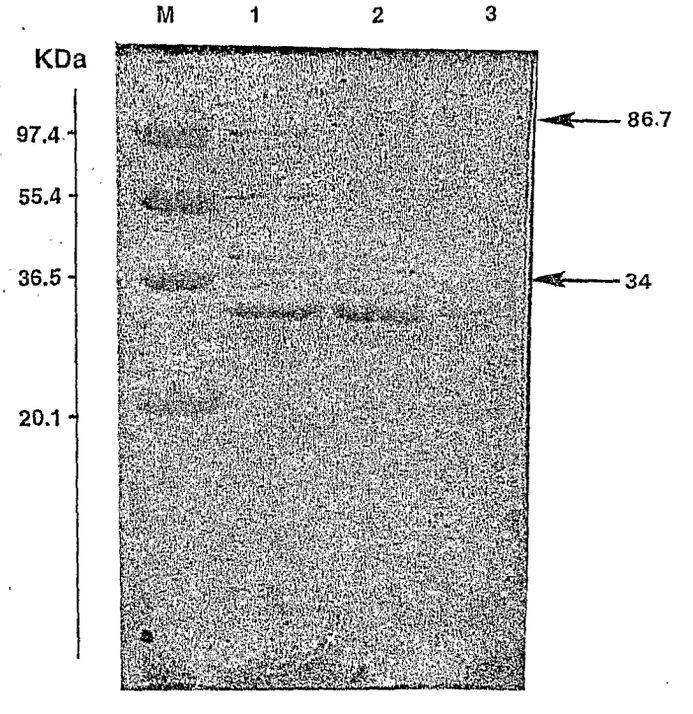

Figure 3. – Polyacrylamide gel profile of *Spodoptera littoralis* granulovirus viral polypeptides. **M:** marker; **1:** MH isolate; **2:** CD isolate; **3:** SII isolate.

Bioassay tests of SlGV were first carried out against second larval instars. The results demonstrated that these premoulting larvae were highly susceptible to the used concentrations. The more concentrated the virus, the higher the percentage of mortality. The lowest percentage of mortality was 47 %, obtained using 4.8×10^8 capsules per ml, and the highest was 79 %, related to 4.8×10^{11} capsules per ml. The LC_{25}, LC_{50} and LC_{90} values are shown in Table I.

Table I. – Calculated LC values in capsules per ml of *Spodoptera littoralis* granulovirus.

	LC_{25}	LC_{50}	LC_{90}
second instar	5.3×10^9	2.8×10^{10}	6.3×10^{11}
fourth instar	2.1×10^{10}	4.5×10^{11}	1.5×10^{14}

The data relative to the fourth-instar larvae demonstrated their susceptibility to SlGV. The highest mortality 91 % was achieved for the fourth instars by the application of 119 OD per ml. The LT_{25} and LT_{50} values were estimated for each of the tested instars and for each dose. Obtained results demonstrated the inverse relation between virus concentration and time required for the mortality (Tables II).

The present results confirm the opportunity of isolating different genotypes of baculoviruses (SlGV) which were yet unsufficiently studied. This fact induces the possibility that newly introduced baculovirus might recombine with indigenous strains (MUNOZ *et al.* 1997) and thus might produce recombinants with unexpected characteristics. This constitutes an important consideration for the introduction of not only genetically engineered baculoviruses, but also new and/or geographically distinct strains as well (VICKERS *et al.* 1991; ABOL-ELA *et al.* 1994, 1996). While genetic modification offers a clear prospect of nucleopolyhedroviruses (NPVs) (BONNING & HAMMOCK 1996), progress along these lines with members of the other baculovirus genus, namely the granuloviruses (GVs), has generally been markedly slower (SMITH & GOODALE 1998).

Table II. – Calculated LT values in days of *Spodoptera littoralis* granulovirus.

	concentration	LT_{25}	LT_{50}
second instar	6.1×10^{13}	23.1	37.4
	6.1×10^{11}	24.2	36.3
	6.1×10^9	48.8	–
fourth instar	6.1×10^{13}	11.5	18.1
	6.1×10^{12}	13.0	21.5
	6.1×10^9	19.0	–

This historical discrepancy is attributable in large part (i) to the availability of insect cell lines permissive for a number of NPV isolates, and (ii) to distinct genotypes in which detailed molecular biological analysis and genetic modification projects can now be easily undertaken. Regarding the different genotypes, they may differentiate in the viability and specificity *in vitro* and/or *in vivo*. In contrast, considerable efforts to develop cell lines which are stably permissive for GV replication have met with little success (CROOK 1991) although LÉRY *et al.* (1997) reported stable maintenance and complete replication of SlGV in *S. littoralis* and *Ph. operculella* cell lines.

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Titre original : Characterization of two egyptian isolates of Spodoptera littoralis (Boisduval) (Lepidoptera : Noctuidae) granulosinus from natural field infestation in Egypt

Titre en Français : (si le document est en langue étrangère) Caractérisation de 2 isolats égyptiens de la granulose de Spodoptera littoralis (Boisduval) (Lepidoptera : Noctuidae), en provenance de champs infestés naturellement, en Egypte

Mots-clés matières : (10 au plus)

Résumé en Français : (150 mots maximum)

Deux isolats de la granulose de S. littoralis ont été isolées de larves mortes provenant de champs de coton non traités, dans les gouvernorats Gharbeia et Sharkeia en Egypte. Les 2 isolats ont été caractérisés par leur profils de restriction. Les profils obtenus diffèrent de celui publié précédemment en provenance de Côte d'Ivoire en Afrique. Les profils polypeptidiques des capsides ont été déterminés et comparé avec celui de leur homologue africain. Des tests biologiques ont été entrepris.

Cotton, Spodoptera littoralis, granulosinus, Egypte, pathogenicity, characterization, virus.

Les titres, mots-clés matières et résumés en Anglais sont indispensables pour les documents destinés à entrer dans les Bases AGRIS et ASFA (Aquat Sciences and Fisheries Abstracts)