Bull. Fac. Agric., Univ. Cairo, 47 (1996):341-354.

NATURAL DNA-RECOMBINATIONS OF *SPODOPTERA LITTORALIS* NPV AMONG FIELD POPULATIONS IN EGYPT

(Received: 2.8.1995)

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ABSTRACT

Six natural isolates belonging to *Spodoptera littoralis* NPV were identified. Using the restriction endonucleases Eco RI and Bam HI, the DNA profiles of each isolate from Giza, Menya, Kaliubeia-Kaha, Kafr El-Sheikh, Gharbeia and Kaliubeia-Tokh were differentiated from that of the reference Egypt-84. DNA electrophoretic profiles of different isolates collected 5 years later were compared to the reference type. The DNA sequence was not changed through three serial passages of viral isolates *in vivo*, while several viral clones were obtained after the 4 th passage.

Key words: DNA profile..Genetic recombination..*Spodoptera littoralis* NPV.

INTRODUCTION

Baculoviruses as the best known group of insect viruses, are widely spread in Egypt and the Mediterranean basin. The nuclear polyhedrosis virus of the cotton leaf worm *Spodoptera littoralis* (S1 NPV) is the most known occluded virus belonging to Baculovirus group since its discovery in Egypt fourty years ago (Abul-Nasr, 1956). Several investigations had been carried out on the pathogenicity, structure and characterization of this virus (Elnagar and Abul-Nasr, 1980; Kislev and Edelman, 1982; Croizier *et al.* 1986; Abol-Ela *et al.*, 1988). The biochemical and molecular identification of a virus

requires a complete nucleotide sequence of the viral genome, and the amino acid sequences of the virus-coded proteins. The closely related viruses may differ only by small regions of distinct base sequences defined by the electrophoretic profile of DNA digested by different endonucleases (Nathan & Smith, 1975 and Croizier 1984).

Spodoptera littoralis NPV is known to be highly recombinant (Abol-Ela *et al.* 1988). Such characteristic was also observed among other NPVs of *Galleria melonella, Trichoplusia ni, Diparopsis watersi and Rachiplusia ou:* and these hosts represent different variants of the baculovirus type *Autographa californica* (Lee & Miller, 1978; Smith & Summers, 1979 and Croizier *et al.* 1980). Other members of the baculovirus group express certain levels of DNA genetic recombinations. The GVs of *Pieris rapae* and *P. brassicae* are considered as two variants of the same virus (Crook, 1981).

Variability among SlNPV isolates in Egypt has been an important point of interest in our investigations for more than five years. The study of the factors inducing the SlNPV-DNA recombinations and the probability of the DNA - sequence changes is essential to understand the natural genetic status of SlNPV and its impact on the environment.

MATERIAL AND METHODS

Host insect:

Two sources of the cotton leaf worm *Spodoptera littoralis* (Boisd) were used:

1- Larval specimens which were collected from naturally infested cotton fields. Each group of collected larvae represents a population from a geographically distinct region, and the different regions are represented by the following Governorates: Gharbeia (GH), Kafr El-Sheikh (KF), Menofeia (MF), Kaliubeia-Kaha (KAL1), Kaliubeia - Tokh (KAL2), Fayoum (FAY), Sakara (GZWa), Giza (GZW), Benisweif (BEN), Menia (MNY), and Assiout (AS). To study the viral nucleic acid recombination among natural populations, larval specimens were first collected during 1986-1988 and then during 1993-1994. The diseased larvae were directly used for purifying viral isolates, while those appeared healthy, were separately kept and reared under observation for any disease induction.

2- A laboratory culture of healthy larvae was maintained for several generations without disease induction. The laboratory insect strain was required for the propagation of some virus isolates as well as for pathological tests.

Isolation and purification of virus strains :

According to the procedure mentioned by Abol-Ela *et al.* 1988, the cadavers or diseased larvae collected from each region were separately examined (for the presence of viral inclusion bodies), then homogenized and highly purified to obtain a pure suspension of SINPV-inclusion bodies collected from a certain region at a certain time. Each suspension of purified polyhedra was labelled and kept under deep freezing.

To avoid any possibility of viral contamination, samples of surviving larvae (naturally collected from infested fields) were kept separately in closed small plastic boxes provided with some cotton leaves from the site of infestation. They were then transported to laboratory and kept under controlled conditions. The appearence of diseased larvae was considered as an isolate, while the pupated larvae were discarded.

Extraction and analysis of viral DNA:

DNA of each viral isolate was thoroughly extracted according to Vlak and Odink, 1979. After digestion by the corresponding endonuclease, a part of the digested DNA was kept as a reference of the respective isolate. Electrophoresis was conducted under 50 V for a duration of 2 hours. The size of DNA fragments was measured in comparison with DNA markers II and III (Abol-Ela, *et al.* 1994).

Successive infection of *S. littoralis* by viral isolates:

Highly diluted doses of the purified polyhedra from certain isolates were used to infect healthy late 4^{th} instar *S. littoralis* larvae. The infectious concentration was estimated as 10 $\mu l/cm^2$ of the diet using a suspension containing $1.4x10^7$ PIBs/ml. The polyhedra obtained from one dead larva of the above mentioned test were used to infect another group of healthy larvae; this procedure continued for 5 to 7 successive series. The percentage of mortality for each viral passage was planned not to exceed 8 %. Searching for viral clones, DNA of each dead larva was separately analysed. The objective of such procedure is to follow the possible changes in the DNA profile (of cloned viral isolates) through experimental passages in healthy larvae.

RESULTS

A- NATURAL ISOLATES COLLECTED DURING 1986-1988:

Six natural isolates of S1 NPV were differentiated by the analysis of their DNA digested by 5 endonucleases (Eco R1, Bam H1, Hind 3, Sac 1 and Sal 1). These isolates represent the following regions: Giza (GZW), Menia (MNY), Kaliubeia-Kaha (KAL 1), Kafr El-Sheikh (KF), Gharbeia (GH), Kaliubeia-Tokh (KAL 2). The comparison with the DNA-profile reference Egypt-84 as well as among different isolates,was examined. The tested isolates could be clearly distinguished as S1 NPV strains. The major differences were demonstrated after the digestion with Eco RI and Bam HI, while the slight differences shown from the digestion with Sal 1, Sac 1 and Hind 3 are not represented.

1- DNA profiles digested with Eco RI:

The profiles of the DNA separately extracted from each isolate and digested by Eco R1 demonstrated the difference among viral strains. The major differences in the sequence of DNA nucleotides were located between fragments B and Q (Fig. 1-A).

In the isolate (KAL 1), supplementary band of 3.8 - 4 kbp is localized between the fragments J and K . This fragment was not observed in any other isolate. The band B of the same isolate is slightly larger in size compared with that of the reference and a confusion of fragments O-P was also observed. As for GZW isolate, the fragment B is missing, while the fragment I is shorter in size than that of the reference Egypt 84. The separation of the bands L- N could not be observed and the band P was lighter and more attached to fragment Q.

The SINPV collected from Kafr El-Sheik (KF) in which the viral DNA was digested with Eco RI, the fragments B, E, F, G, O M and Q were smaller in size than those of the reference. The isolate (GH) expressed a net variability concerning the fragment group H, I and J.

Comparison with (MYN) isolate indicated some diferences in the fragment molecular weight and co-migration: the band B was slightly heavier while J seemed to be distinctly smaller. A co-migration was observable among bands O & P. Except that the fragments B was missing, the isolate (KAL2) was almost similar to the isolate (GZW).

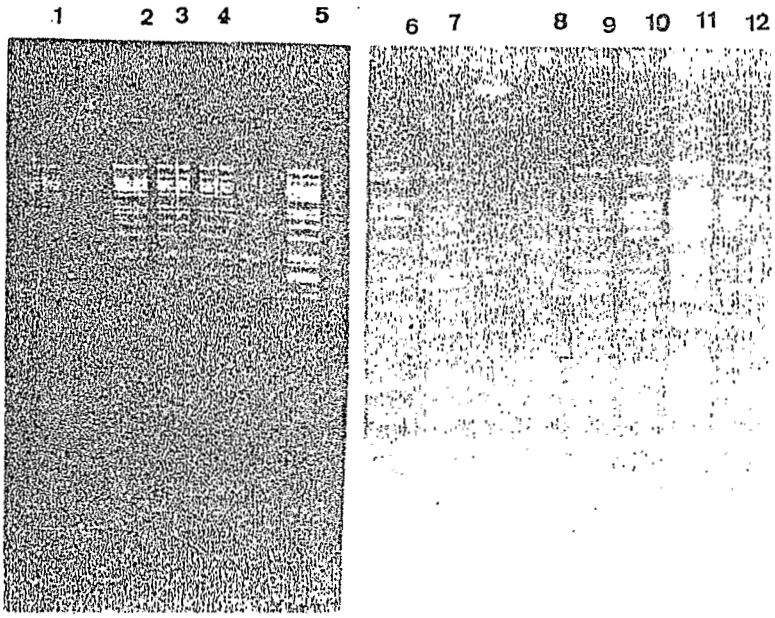

Fig. 1 (A)

Fig.1(B)

Fig. 1 : DNA profile of different isolates of Sl NPV collected during 1986-1988.

A - Digested by Eco R1:
1- Egypt 84 2- KAL 2 3- MNY 4- MYN passage 5- KF 6- Ref. Morocco 7- KF 8- MNY 9- GZW 10- KAL2 11- KAL1 12- Egypt 84.

B - Digested by Bam H1:
1- Egypt 84 2- KAL 2 3- KAL1 4- KF 5- Ref. Morocco

2- DNA profiles digested with Bam III:

As shown in Fig. 1-B, important differences were found in the comparison with the reference Egypt 84 when the DNA of different viral isolates was digested by Bam HI. A supplementary band, located between B and C, was observed in the DNA- profile of GH and KAL2. This band was located between C & D in MNY and GZW isolates. Fragments G and H were heavier in molecular weight among KF and MNY. The same fragments were lighter (migrated more rapidly) in the gel of KF, GH, GZW and KAL 1.

B- DNA PROFILES OF VIRAL ISOLATES COLLECTED DURING 1993-1994:

The research on DNA sequence structure of viral isolates infecting natural *S. littoralis* larval populations was conducted through two directions:

- Determination of the DNA profiles of viral isolates collected during 1993 from the same localities. The DNA fragment sequences were compared with those of the specimens collected during 1986-88. In this respect, two characteristic isolates were recommended in order to follow the expected evolution of the viral DNA : KAL 1 (representing Delta region) and MYN (representing Upper Egypt region).

- Determination of the DNA- electrophoretic profiles of other viral isolates originated from new localities and comparing them with the reference Egypt 84.

1- DNA-enzymatic profiles of KAL 1 and MYN viral isolates compared with those of 1986-1988.

When compared with the isolate KAL 1 84-86, the DNA-profile of KAL 1 93-94, digested with Bam HI, showed two missing bands at the levels of D and H (13.8 and 6.5 kbp). Using Eco RI as a digestion endonuclease for the viral DNA of the isolate KAL 1, the results demonstrated that the supplementary band situated between J and K in KAL 1-86 (compared with the reference), is not present in the DNA profile of KAL 1-93. This missing fragment is estimated by 3.8 - 4 kbp (Figs 2,4 and 5).

In the MYN isolate - DYN digested by Bam H1, the fragment D (14.5-15 kbp) disappeared, while a supplementary band (6.8-6.4 kbp) appeared just over fragment G. Changes in the molecular weights of the fragments H and I, which became shorter in size, were also noted in the DNA-profile of isolate MYN 93-94.

2- **The status of the DNA-profile of other viral isolates collected in 1993-94 compared with the reference type Egypt-84:**

Other specimens of S1 NPV were collected during 1993-94 from natural populations infesting cotton fields. The viral specimens were collected from: Fayoum (FAY), Sakara (SK), Menofeia (MNF), Benisweif (BEN) and Assiout (AS). The extracted DNA of each isolate was digested with Eco RI and Bam HI, and the endonuclease Eco R5 was additionally used for comparison in special cases.

As demonstrated in Figs. 2,4 and 5 , DNA enzymatic profiles of all collected isolates, digested with Eco RI, were identical and the fragment sequences presented the same molecular weights. DNA-fragment sequences of all above mentioned isolates were also identical to the reference Egypt-84. The digestion of extracted DNA by the endonuclease Bam HI indicated that all isolates were also closely related to the reference profile Egypt-84. The minor differences observed with this endonuclease were limited at the level of fragments G (slightly heavier in the isolates) and H (heavier in the isolates).

C-THE IMPACT OF SUCCESSIVE INFECTION ON THE DNA PROFILE OF VIRAL ISOLATES:

Certain isolates of S1 NPV were used for successive infections against their respective host. The objective of this laboratory experiment is to follow the changes in the DNA profiles through serial passages of the same viral isolate in a healthy host, as well as the determination of the nature of viral clones.

Three isolates were chosen for this experiment: GZW, KAL 1 and MYN. Bam HI was used for DNA analysis.

The sequences of DNA-fragments of the tested viral isolates were identical during the first three passages. The fourth passage of serial infection resulted in four different viral clones for GZW. The DNA-profile was different among viral clones, as well as between the isolates.

As shown in Fig. 3, the clone number 4 in GZW is characterized by the absence of fragments A, B and C which are found in KAL 1, while only the band C is found in the clone GZW number 3. The profile of the clone GZW number 2 contains the bands A, B and C, in addition to 2 supplementary bands. The clone GZW number 1 is characterized by the different migration rate of fragments A, B and C.

Fig. 2 (A)

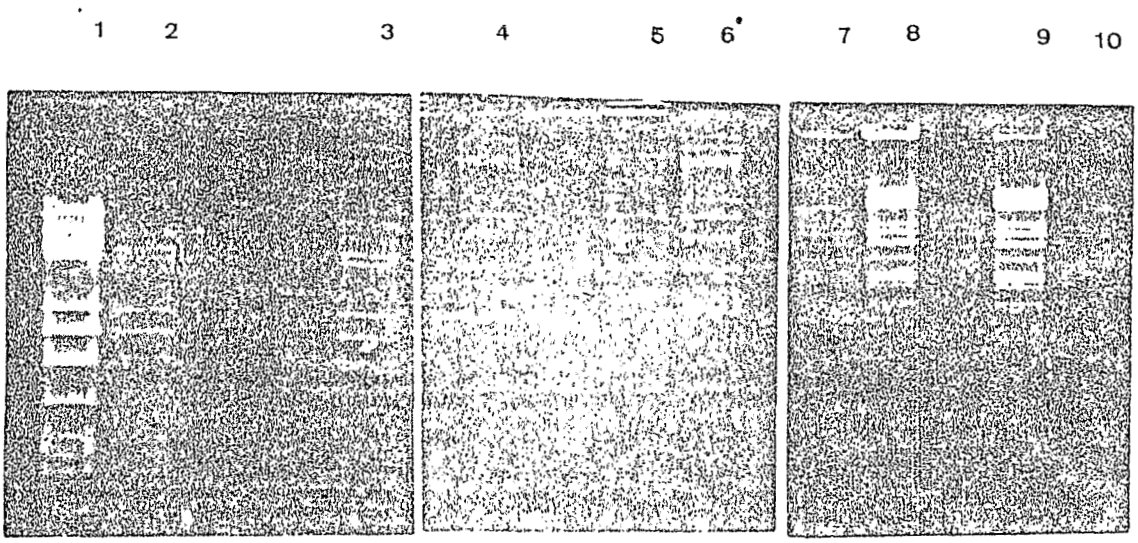

Fig.2 (B)

Fig. 2 : DNA profile of different isolates of Sl NPV collected during 1993-1994.

A - Digested by Eco R1:
1- AS 2- GZW 3- KALI.

B - Digested by Bam H1:
1- AS 2- GZW 3- KALI 4- FAY 5- MNY 6- KALI 7- BEN 8- GZW 9- AS
10- FAY .

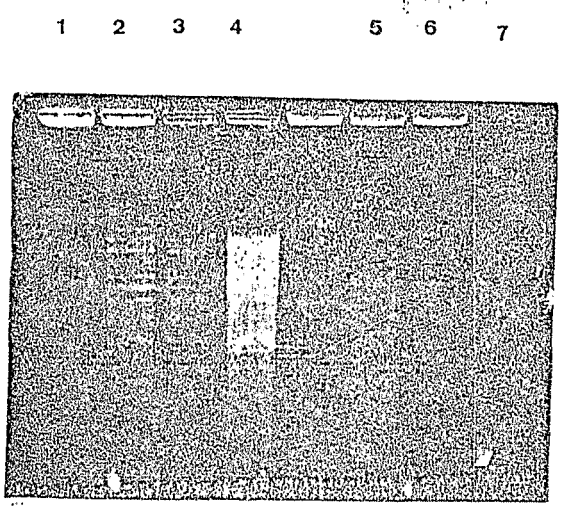

Fig. 3 : Viral clones from GZW and KAL1 isolates after four passages *in vivo* .
1- GZW (clone 4) 2- KAL1 (clone 1) 3- KAL1 (clone 4) 4- KAL1 (clone 7) 5- GZW (clone 3) 6- GZW (clone 2) 7- GZW (clone 1).

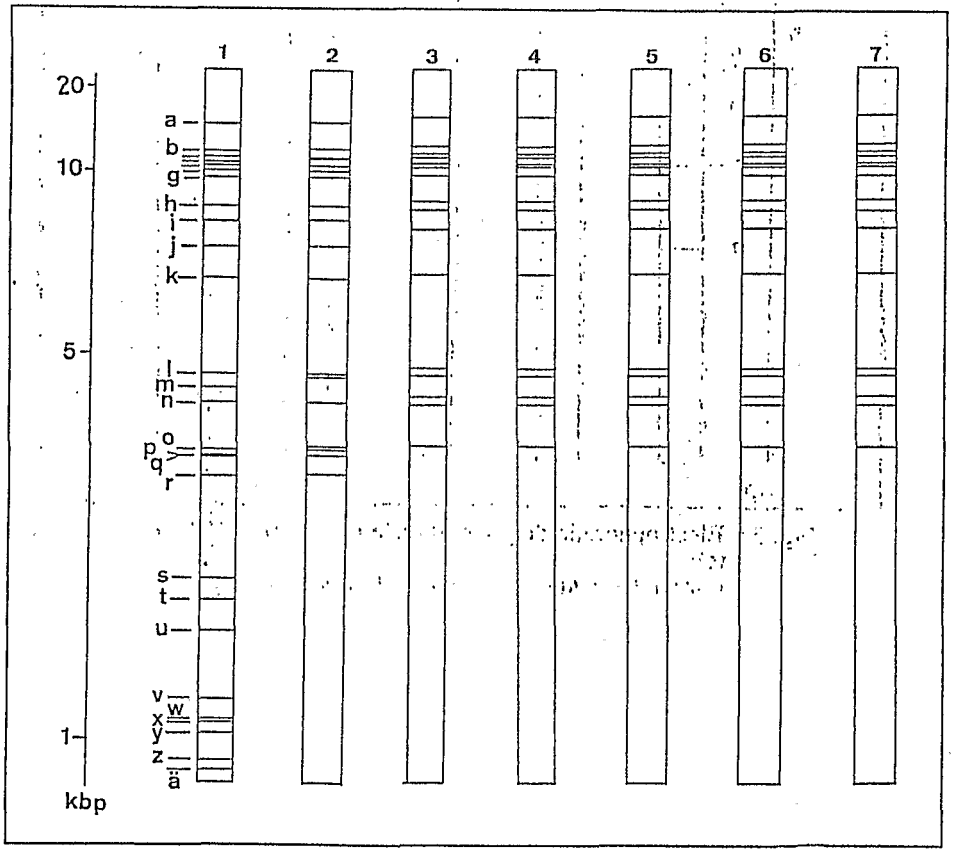

Fig. 4 : Electrophoretic diagram of different isolates of SI NPV digested by Eco R1:
1- SI NPV- Type B. 2- MYN 86 3- MYN 93 4- FAY 93 5- MF 93
6- GZWa 93 7- GZWb 93.

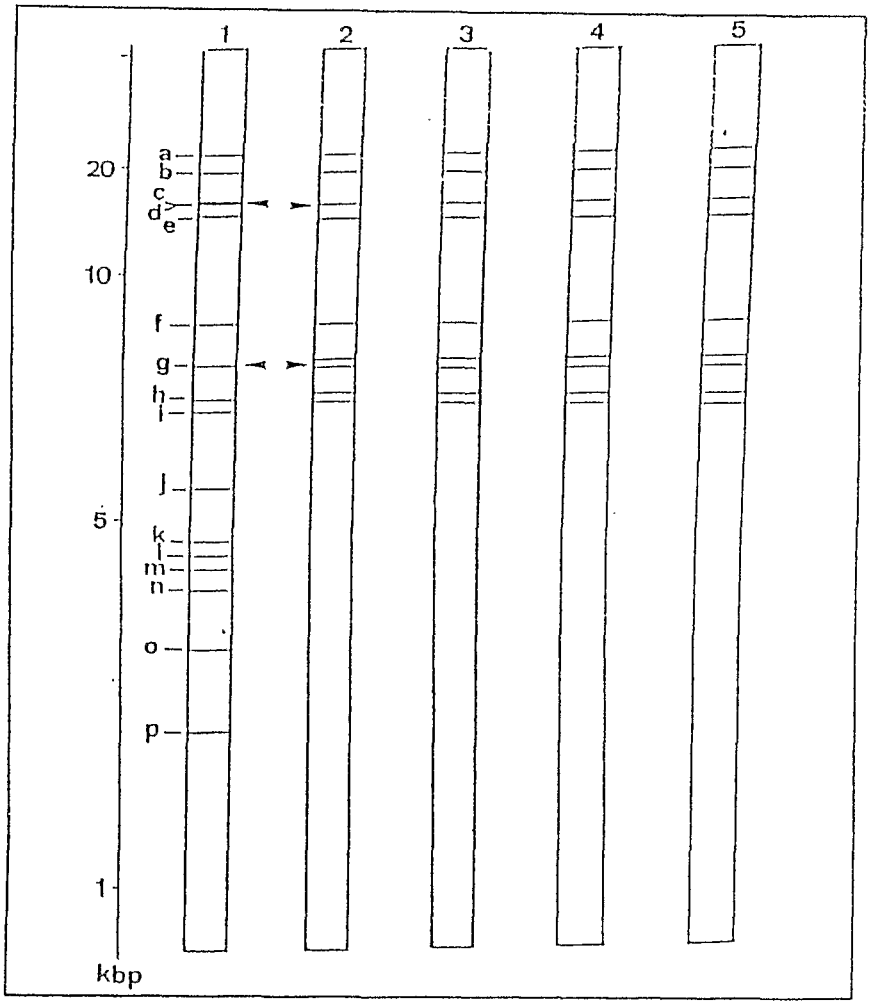

Fig. 5 : Electrophoretic diagram of different isolates of Sl NPV digested by Bam H1:
1- MNY 86 2- MYN 93 3- GZW 93 4- BEN 93 5- MF 93.

DISCUSSION

Spodoptera littoralis NPV isolates, identified in the present investigation, belong to type B. The type A, which is more interesting as an expression vector for certain important genes, has not been identified in Egypt so far (Kislev and Edelman, 1982). The characteristic of S1 NPV as a highly recombinant virus is not limited in the apparent fragility of its DNA stability, it could be extended to its role as a genetically dynamic microogranism in the eco-system.

Classification of distant isolates based on the use of DNA homology techinques holds much promise. The experience with the polydisperse DNA viruses has shown that morphological data alone may not be sufficient for reporting new virus isolates. Information on the size and conformation of the viral DNA, together with a restriction endonuclease profile, is required (Lee and Miller, 1978; Jewell and Miller, 1980; Miller *et al.* 1980).

Since identical profiles do not necessarily suggest complete identity, the use of a battery of enzymes is recommended to ensure that the position of the restriction sites as well as the size of the fragments are identical. The major limitation of REN analysis is that the results are difficult to quantify. It is essential that DNA is completely digested, because incomplete digestion will yield profiles that do not follow the strict resolution between band intensity and fragment size and will not be representative of the analysed DNA. Strictly quantitative studies of DNA homology would require sequencing of the entire genome or the use of reassociation kinetics. This technique was utilized by Kelly (1977) to study the relationship among the DNAs of viruses from *Spodoptera* spp.

The existence of different S1 NPV isolates in some distinct regions in Egypt excludes the identification of a unique type of S1 NPV. The later diagnosis of this virus shoud be referred by its DNA-type. Otherwise, the S1 NPV is considered as a localized viral population. However, the appearence, absence or comigration of the major DNA fragments (A, B, C, D and E) reflects the importance of the genetic recombination within the viral population (MYN, KAL and GZW isolates).

The multiplication of the viral isolates through their host larvae did not change the sequence of DNA-fragments. However, the laboratory trial for cloning GZW, KAL 1 and MYN isolates *(in vivo)* produced different viral clones. For the time being and till the confirmation of these results *in vitro* (on-going research), this phenomenon cannot be explained. However, O'Reily and Miller (1989) stated that the region of *Autographa californica* NPV-

genome containing the egt gene spacing of 8.4 -9.6 map units could bring our attention to a hyper mutable region in serially propagated viruses.

Genetic recombination among S1 NPV populations may be conditioned by certain environmental or biological factors such as the existence of other baculoviruses (Croizier, *et al.*, 1980 and 1988; Smith and Crook, 1988). Viral isolates collected five years later, from the same locations and from new sites, indicated that the sequence of the DNA - fragments, for all tested isolates, was oriented to the reference type (Egypt-84), a continous identification of viral isolates is required to confirm the long-term tendency of viral recombinations. The variability of S1 NPV isolates and the subsequent effect on the viral pathogenicity are important considerations for the viral biocontrol agent.

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