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ISOLATION OF DENSOVIRUS AND PICORNAVIRUS FROM NATURAL FIELD POPULATIONS OF *Spodoptera littoralis* BOISD. (LEP. NOCTUIDAE) IN EGYPT.

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ABSTRACT

Two natural viral strains belonging to the Parvoridae (Densovirus) and the Picornaviridae (Picornavirus) families were isolated from larvae of the Egyptian cotton leafworm *Spodoptera littoralis.* The strains were sampled from natural infestation of cotton field in Kafr El-Sheikh Governorate in may 1997. Electron microscopy observations revealed the presence of icosahedral particles with 22 nm in diameter for the viral capside related to the Densovirus (DNV), and 29 nm for the second one as a Picornavirus (PV). For the DNV four capsid proteins of 91, 63, 53 and 47 KDa have been separated, and were undifferentiated from that of the unique published Densovirus isolate from Egypt, the *Mythimna loreyi* DNV(*Ml*DNV). PV capsids contain three major structural proteins of 30, 32 and 33 KDa resembling the Drosophila C virus (DCV), a very well known Picornavirus of insect. The DNV genome consists of a single stranded DNA molecule of size 5. 95 Kb. It was characterized using 12 restriction endonucleases. The DNA restriction profiles were identical with these of *Ml*DNV. The PV contain a single stranded RNA genome of size 9. 4 Kb as the different reported isolates of DCV.

Using rabbit antisera, immunological comparison revealed a complete homology between the DNV and *Ml*DNV, as well as, the PV isolated from *S. littoralis* shows a complete serological identity with the DCV.

This results suggest that we can registrated the DNV isolated from *S. littoralis* as a strain of *Ml*DNV and the small RNA virus as an Egyptian strain of DCV.

Larvae of *S. littoralis* reared on artificial diet in the laboratory were susceptible to these two viruses.

Keywords: cell line Sl52, Cotton leafworm, Densovirus, Parvoviridae, Picornaviridae, Picornavirus, *Spodoptera littoralis*.

INTRODUCTION

Different invertebrate viruses were already isolated from the Egyptian cotton leafworm *Spodoptera littoralis* Boisduval in Egypt. Firstly two member of the family Baculoviridae were detected. A Nucleopolyedrovirus (*Sl*NPV) was isolated fourty three years ago by Abul-Nasr, (1956), then a Granulovirus (*Sl*GV) was isolated from diseased larvae fifteen years ago at Bouaké in

Côte d'Ivoire and characterized in Egypt through its biological, immunological and biochemical properties by Abol-Ela *et al.,* (1994) and Abd-Alla *et al.,* (1997). Recently two new strains of the *Sl*GV, Egyptian isolates, were characterized from Gharbeia and Sharkeia governorates (Khamiss *et al.,* 1999). Finally an unusual free virus, firstly recorded among lepidopterous as a Bunyavirus (*Sl*BV) was isolated by Abol-Ela *et al.,* (1995). Since now, no viral strains belonging to the Parvoridae (Densovirus) and the Picornaviridae (Picornavirus) families were recorded from *S. littoralis*.

Densovirus share many properties with vertebrates Parvovirus, but their genome organization and their expression strategy are completely different (Bergoin and Tijssen, 1998). Therefore two subfamilies are recognized within the Parvoviridae family, the Densovirinae and the Parvovirinae (Tijssen and Bergoin, 1995). Despite their high virulence, their infectivity and their polyspecificity towards a broad host-range, the use of Densonucleosis Viruses (DNV) as biological pesticide has not yet been investigated in detail because of non achievement of inocuity tests. Nevertheless, the successful control of at least two insect pests of oil palm by DNVs was reported in Colombia (Genty and Mariau, 1975) and in Côte d'Ivoire (Fediere *et al.*, 1986) against respectively *Sibine fusca* (Stoll) and *Casphalia extranea* (Walker). A Densovirus isolated in Egypt from the maize worm *Mythimna loreyi* Duponchel, designated as *Ml*DNV (Fediere *et al.*, 1995), is polyspecific and infects experimentally a large number of Egyptian Lepidopterous pests as well as *S. littoralis*, the European corn borer *Ostrinia nubilalis* Hubner, the maize pink borer *Sesamia cretica* Lederer, the rice purple line borer *Chilo agamemnon* Bleszynski, the cotton pink bollworm *Pectinophora gossypiella* Saunders and *Galleria mellonella* Linnaeus (Fediere, 1996). In view of the important property of host range for biological control, a complete homology between *Ml*DNV and a natural strain from *S. littoralis* could approve the natural polyspecificity of *Ml*DNV in the field.

In recent years the number of descriptions of small (less than 40 nm in diameter) nonoccluded RNA-viruses from insects has increased steadly although few have yet to be placed within a defined taxonomic framework. Execptions to this are members of the Cricket Paralysis Virus (CrPV) and Drosophila C Virus (DCV) complex, placed within the Picornaviridae family, as Picornavirus (PV) of insects (Christian and Scotti, 1994).

This work is considered as a part of the program concerning the inter-relationship between the different viruses isolated from *S. littoralis* and their host, and the biodiversity of the viral strains regarding as well as the geographical distribution of this pest in Egypt and the host-crops.

In this paper, we describe two new viral strains from *S. littoralis*. The relevant research concerns the virus populations analysis among naturally infected *S. littoralis* from the fields and the laboratory rearing, and the identification of the viral isolates.

MATERIALS AND METHODS

The Virus isolates

The strains of DNV and PV of *S. littoralis* were sampled as well as, from larvae collected during a natural infestation of cotton field in Kafr El-Sheikh Governorate in may 1997, and from natural dead-larvae of the mass-rearing of this pest in the laboratory during the year 1998.

The strain of *M. loreyi* DNV designated as *Ml*DNV was the original strain propagated for several years in our laboratory (Fediere *et al.*, 1995). The strain DCV Picornavirus, the rabbit immumserum anti-DCV and the cell lineSl52 were obtained from Dr. F. X. Jousset, Saint-Christol-Les-Ales, France (personal communication).

Virus purification

The infected larvae were homogenized in Tris (0.05M)-SDS (0.06%) Buffer, pH 7.8. After filtration through gouze textile and clarification (10,000 g for 5 min), the virus was concentrated by high speed centrifugation (Ti 55 Beckman rotor, 35.000 rpm for 2 h). The pellets, resuspended in Tris (0.05M)-Buffer, pH 7.8, were dispersed by ultrasonication and then clarified (10,000 g for 5 min). The resulting supernatant, containing virus particles, was layered onto sucrose gradient (15-45%) prepared in Tris-Buffer and centrifuged at 120,000 g during 2 h 30, then the band of virions was collected and washed using Tris-Buffer. The virions were pelleted by centrifugation (Ti 55 Beckman rotor, 35.000 rpm for 2 h) and then, the virus was resuspended in 2 ml of Tris-Buffer, the concentration of the final suspension was measured on the spectrophotometer at 260 nm.

Electron microscopy

Purified viral suspension was negatively stained in 2 % uranyl acetate, pH 7.4 and the grids were examined through electron microscope.

Serology

Double diffusion tests in 1% agar were done using antisera to DCV and to *Ml*DNV and the different viral strains.

ELISA tests

For detecting the viral proteins, ELISA was conducted using the direct method. Specific conjugate was elaborated by extracting globuline from total rabbit immunosera anti-DCV and anti-*Ml*DNV. IgG-enzyme conjugate was prepared and used at concentration of 1/1000. The same dilution was used for the globuline. For the samples several dilutions of 1/10, 1/100 and 1/1000 were used.

Electrophoresis of the viral proteins

Molecular weight and number of proteins were assessed by comparing their electrophoretic mobilities in 12% polyacrylamide gel

according to Fediere *et al.* (1995) with those of standard marker proteins (Pharmacia).

Extraction and analysis of viral nucleic acid

The extraction of nucleic acid from the purified virus was carried out using the procedure of Fediere *et al.* (1993). The nucleic acid precipitate was resuspended in Tris (15mM)-EDTA (1mM)-Buffer, pH 7.5. The concentration of the final suspension was measured on the spectrophotometer at 260 nm.

To determine whether the nucleic acid nature is DNA or RNA, the two samples (1 ug in 10 ul) were treated with DNAse and RNAse (0.1 ug), then analyzed by electrophoresis on a 1% agarose gel.

Estimation of RNA-genome size

The RNA-genome size was estimated by electrophoresis in formaldehyde denaturing 1% agarose gel as recommended by Maniatis *et al.* (1982), using standard molecular weight RNA marker (Promega).

Restriction enzyme digestion and electrophoresis of the viral DNA

The DNV-DNA was digested with the 12 following endonucleases: Bam HI, Bgl II, Eco RI, Hae II, Hae III, Hha I, Hinc II, Hind III, Hpa I, Pst I, Sca I, Xba I, under conditions specified by the supplier (Boehringer). The digested fragments were analysed by electrophoresis on horizontal 1% agarose gel. The gel was visualised and photographed on a UV transIluminator. The size of the DNA fragments was estimated by comparison with standard marker DNA (Boehringer).

DNA probe

A DNA probe prepared from the total extract DNA of the *MI*DNV was used. The digoxygenin-labelled DNA probe was applied according to the protocol recommended by the supplier (Boehringer). The same protocol was applied for the "dot blot" hybridization technique to detecte homology between the *MI*DNV-DNA and the DNV-DNA of *S. littoralis.*

RESULTS AND DISCUSSION

Examination of purified viral suspension by electron microscopy revealed the presence of large number of icosahedral non-enveloped particles of 22 nm and 29 nm in diameter (Fig. 1) resembling the Densovirus (DNV) of family Parvoviridae and the Picornavirus (PV) of family Picornaviridae respectively.

Electrophoresis of viral proteins for the DNV revealed, four capsid polypeptides of 91(VP1), 63(VP2), 53(VP3) and 47(VP4) KDa, with the VP4 as a major band (Fig. 2). These values are undifferentiated from that of the unique published Densovirus isolate from Egypt, the *MI*DNV). PV capsids contain three major structural proteins of 33(VP1), 32(VP2) and 30(VP3) KDa (Fig. 2), resembling those of the DCV, a very well known Picornavirus of insect.

Extracted nucleic acid of the two samples digested by both DNAse and RNAse and then electrophoresed revealed the resistance to RNAse, prove the DNA nature for the DNV, and the resistance to DNAse, prove the RNA nature for the PV.

The DNV genome consists of a single stranded DNA molecule of size 5. 95 Kb. It was characterized using 12 restriction endonucleases and the DNA restriction profiles were identical with these of *Ml*DNV (Fig. 3 and Table 1). The PV contain a single stranded RNA genome of size 9. 4 Kb as the different reported isolates of DCV.

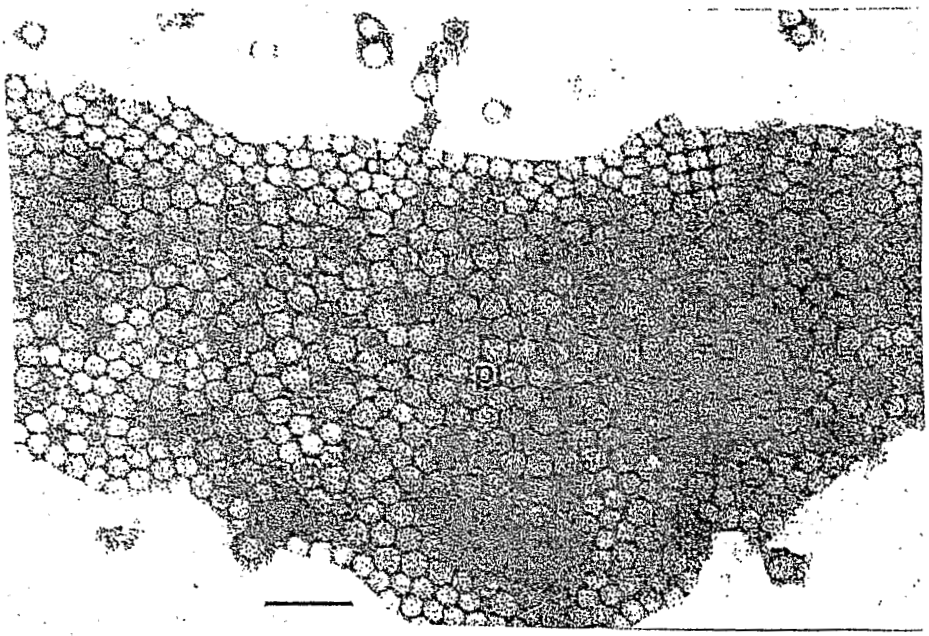

Fig. (1). Electron micrograph of a purified viral suspension of mixed *S. littoralis* Densovirus (d) and Picornavirus (p). Bar represents 100 nm.

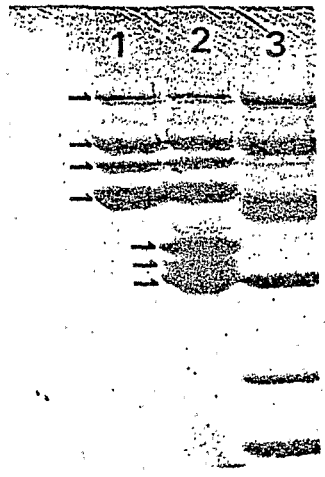

Fig. (2). Electrophoretic analysis of purified viral DNV and PV particles from *S. littoralis*. The polypeptides were separated by 12 % SDS-PAGE. Lane 1: *Ml*DNV; Lane 2: mixed *S. littoralis* DNV and PV; Lane 3: Proteins markers (94, 67, 43, 30, 20.1 and 14.4 KDa).

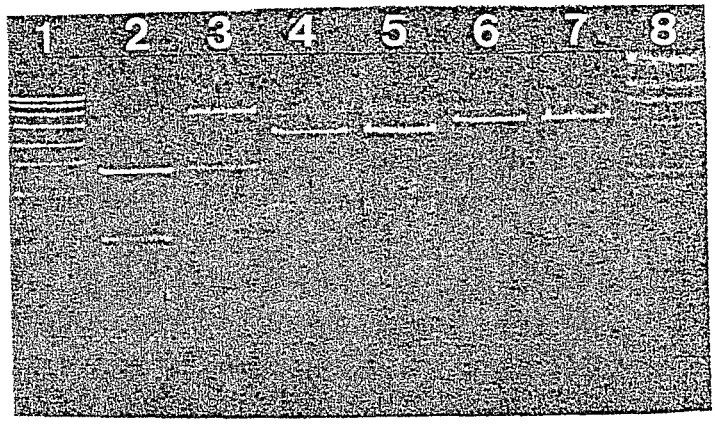

Fig. (3) : Restriction endonuclease profiles of digested viral DNA from *S. littoralis* DNV and *Ml*DNV in 1 % agarose gel. Lane 1: Standard molecular weight DNA marker VII (Boehringer); Lane 2: *Ml*DNV-DNA digested by Hha I; Lane 3: *S. littoralis*DNV-DNA digested by Hha I; Lane 4: *Ml*DNV-DNA digested by EcoR I; Lane 5: *S. littoralis*DNV-DNA digested by EcoR I; Lane 6: *Ml*DNV-DNA digested by Hind III; Lane 7: *S. littoralis*DNV-DNA digested by Hind III; Lane 8: Standard molecular weight DNA marker II (Boehringer).

Table (1): Number and size (in Kb) of different restriction fragments generated by 12 endonucleases from the *S. littoralis* DNV-DNA

Enzyme	A	B	C	D	E	F	G
Bgl II	4.50	1.45					
Pst I	3.85	2.1					
Bam HI	5.39	0.28	0.28				
Hinc II	4.88	0.57	0.50				
Hpa I	3.84	1.18	0.47	0.37	0.09		
Eco RI	4.05	1.59	0.31				
Hind III	4.74	0.92	0.16	0.09			
Hae III	1.64	1.14	0.99	0.69	0.47	0.48	0.39
Hae II	3.35	2.03	0.57				
Xba I	2.57	1.88	0.77	0.71			
Sca I	3.28	1.24	0.71	0.49	0.13		
Hha I	2.51	1.06	0.95	0.43	0.33	0.32	0.19

Using rabbit antisera, immunological comparison reveale a complete homology between the DNV and *Ml*DNV, as well as, the PV isolated from *S. littoralis* shows a complete serological identity with the DCV.

It will be interresting to clone the genomes of these two new strains of *S. littoralis* virus into bacteria using DNA and cDNA techniques and study the sequence homology with *Ml*DNV and DCV.

These results suggest that, since the complete sequence homology was achieved, we can state that registrated the DNV isolated from *S. littoralis* as a new strain of *Ml*DNV. This fact illustrates the wide host range of this virus, and the small RNA virus of *S. littoralis* as the first Egyptian strain of DCV.

It is important to note that during several assays performed positively to multiply *in vitro* the *Ml*DNV by infection and transfection of a cell line from *S. littoralis* (Sl52), two small viruses of 22 and 29 nm were also isolated (unpublished data). The quantity of these viruses was not quite enough to facilitate the detailed studies. It will be interesting to compare these two viruses chronically infecting the cells of this culture from the host, with the Densovirus and the Picornavirus isolated *in vivo*.

The confirmation of the natural polyspecificity of *Ml*DNV in the field complete its biological characterization and would provide investigators a more completely known pathogen for use as microbial agent against *S. littoralis* larvae on cotton. It is possible that *Ml*DNV may represent an important complementary agent alongside with *Sl*NPV and *Sl*GV in the IPM programmes of the pest, as the use of a Granulovirus (*Sc*GV) for microbial control of the maize pink borer *S. cretica* in Egypt (Fediere *et al.*, 1997). Whereas, concerning the Picornaviruses of insects, unfortunately, for severals reasons, they were not accepted for direct use in biological control programs.

REFERENCES

Abd-Alla, A.M., El-Sheikh, M.A.K., Abol-Ela, S., Fediere, G., Giannotti, J., El-Sharaby, A.M.F. (1997). Laboratory bioassay tests and host range of the Granulosis virus of *Spodoptera littoralis*. Bull. ent. Soc. Egypte, Eco. Ser., 24: 22-32.

Abol-Ela, S., Fediere, G., Nour-El Din, A., Kamiss,O. and Salah,M. (1994). Restriction endonucleases and diagnosis of the granulosis virus isolated from *Spodoptera littoralis* Boisd. in West Africa and multiplied in Egypt. Bull. Fac. Agric. Univ. Cairo, 45: 919-932.

Abol-Ela, S., Khamiss, O., Louis, C., Fediere, G., Monsarrat, A., Giannotti, J. (1995). Natural infection of the cotton leafworm *Spodoptera littoralis* by an unusual free virus, firstly recorded among lepidopterous insect. Proc. 5 th IOBC European Meeting, Poznan, Poland, August 27 - September 1.

Abol-Ela, S., O. Khamis, J. Giannotti, G. Fediere, X. Lery and Belal M. (1996). Natural DNA-recombinations of *Spodoptera littoralis* Npv among field populations in Egypt. Bull. Fac. Agric. Univ. Cairo. 47: 341-354.

Abul-Nasr, S. (1956). Polyhedrosis- virus disease on cotton leafworm, *Prodenia litura* F. Bull. Soc. ent.Egypte. 40: 321-332.

Bergoin, M. and Tijssen, P. (1998). Biological and molecular properties of Densoviruses and their use in protein expression and biological control. In: The insect viruses. Miller, L.K. and Ball, L.A. (Eds). Plenum Press, New York, USA, 141-169.

Christian, P. D. and Scotti, P. (1994). A suggested taxonomy and nomenclature for the the Cricket Paralysis and Drosophila C Virus complex. Journal of Invertebrate Pathology. 63: 157-162.

Fediere, G. (1996). Recherches sur des viroses de Lepidopteres ravageurs de cultures perennes en Cote d'Ivoire et de cultures annuelles en Egypte. Doctorat d'Etat, Universite de Montpellier II, 204 pp.

Fediere, G., Monsarrat, P., Mariau, P. and Bergoin, M. (1986). A Densovirus of *Casphalia extranea* (Lepidoptera: Limacodidae): characterization and use for biological control. In: Fundamental and Applied Aspects of Invertebrate Pathology. Samson, R.A., Vlak, J.M. and Peters, D. (Eds). Society of Invertebrate Pathology, Wageningen, The Netherlands, 705.

Fediere, G., Taha, A., Abol-Ela, S, Lery, X., Zeddam, J.L., Veyrunes, J.C. and Giannotti, J. (1993). Mise en évidence d'un virus de granulose chez *Sesamia cretica* Led. (Lepidoptera Noctuidae) principal ravageur du maïs d'Afrique du Nord-Est: Caractérisation de l'ADN génomique et diagnostic viral. Comptes Rendus Hebdomadaires de l'Académie des Sciences, Paris. 316: 1350-1354.

Fediere, G., El-Sheikh, M.A.K., Abol-Ela, S., Salah, M., Masri, M. and Veyrunes, J.C. (1995). Isolation of a new Densonucleosis Virus from *Mythimna loreyi* Dup. (Lep. Noctuidae) in Egypt. Bull. Fac. Agric.Cairo. 46, (4): 693 - 702.

Fediere, G., El-Sheikh, M.A.K., Semeada, A.M., El-Hefny, A., Masri, M. and El-Sherif, S. (1997). Production and field evaluation of a Granulosis Virus for control of *Sesamia cretica* Led. (Lep. Noctuidae) in maize field in Egypt. Journal of Applied Entomology. 121: 293-296.

Genty, P.and Mariau, D. (1975).Utilisation d'un germe entomopathogène dans la lutte contre *Sibine fusca* (Limacodidae). Oléagineux. 30: 349-354.

Jousset, F.X., Bergoin, M. and Revet, B. (1977). Characterization of the Drosophila C Virus. Journal of general Virology. 34: 269-285.

Khamiss, O., Giannotti, J., Fediere, G., Lery, X. and Nour-El-Din, A. (1999). Characterization of two Egyptian isolates of *Spodoptera littoralis* Boisd. (Lepidoptera : Noctuidae) Granulovirus isolated from natural field infestaion in Egypt. In: Applied Biological Control. in Mediterranean Countries. Canard, M. and Beyssat-Arnaouty, V. (Eds), Toulouse, France, 147-152.

Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982). Molecular cloning: a laboratory manual. New-York, Cold Spring Harbor Laboratory (Eds). 580 p.

Tijssen, P. and Bergoin, M. (1995). Densonucleosis Viruses constitute an increasing diversified subfamily among the Parvoviruses. Seminar in Virology. 6: 347-355.

عزل فيروس دينسو (Densovirus) وفيروس بيكورنا (Picornavirus) من تعدادات حقلية طبيعية لدودة ورق القطن في مصر.

جبيل فيدير – محمد الشيخ * – أميمة خميس – مها مصري – مجيب صالح

معمل فيروسات الحشرات و * قسم الحشرات الإقتصادية والمبيدات – كلية الزراعة – جامعة القاهرة – الجيزة.

عزلت سلالتان فيروسيتان طبيعيتان تنتميان لعائلات Parvoviridae (Densovirus) ، Picornaviridae (Picornavirus) من يرقات دودة ورق القطن المصرية *Spodoptera littoralis* . أجري جمع عينات السلالتين من الأصابات الطبيعية في حقل قطن بمحافظة كفر الشيخ خلال مايو (1997).

كشفت الملاحظات باستخدام الميكروسكوب الإليكتروني عن وجود جزيئات ذات عشرون وجها" (icosahedral) يبلغ قطر الكبسولة الفيروسية ٢٢ نانومتر في فيروس الدينسو Densovirus (DNV) ، ٢٩ نانومتر في فيروس البيكورنا Picornavirus.

تم فصل أربعة بروتينات لكابسد فيروس الدينسونيوكليز (DNV) أوزانها الجزيئية 47,53,63,91 كيلو دالتون ، حيث كانت غير مختلفة عن تلك الخاصة بفيروس الدينسو الفريد المعزول من مصر والمنشور تحت أسم فيروس دينسونيوكليز دودة الذرة *Mythimna loreyi* DNV (*Ml* DNV).تحتوي كابسدات فيروس البيكورنا (PV) علي ثلاث بروتينات تركيبية رئيسية أوزانها الجزيئية 33,32,30 كيلو دالتون مشابهة لفيروس C الدروسوفلا (DCV) وهو فيروس بيكورنا حشري معروف جيدا. يتكون جينوم فيروس الدينسو DNV من جزئ حمض نووي من نوع الـ DNA مفرد الخيط ذو حجم 5.95 كيلو قاعدة . وقد تم تشخيصه بإستخدام إثني عشر إنزيما" من إنزيمات القطع المحددة . كانت البصمات المحددة للحمض النووي متطابقة identical مع تلك الخاصة بفيروس *Ml* DNV. يحوي فيروس البيكورنا على جينوم حمض نووي من نوع الـ RNA ذو حجم 9.4 كيلو قاعدة كما هو الحال بالنسبة لمختلف العزلات التي سجلت لفيروس الـ DCV.

كشفت المقارنة المناعية باستخدام السيرم المضاد للأرنب عن التماثل الكامل Complete homology بين فيروس الـ DNV ، فيروس دينسو نيوكليسز دودة الذرة *Ml*DNV ، علاوة علي أن فيروس البيكورنا المعزول من حشرة دودة ورق القطن قد أظهر تماثل سيرولوجي كامل Complete serological identity مع فيروس الـ DCV.

تقترح هذه النتائج أنه يمكننا أن نسجل أن فيروس الدينسو DNV المعزول من حشرة دودة ورق القطن هو سلالة لفيروس دينسو دودة الذرة *Ml*DNV وفيروس الـ RNA الصغير هو سلالة مصرية لفيروس الـ DCV.

وقد أظهرت يرقات دودة ورق القطن المرباة على البيئة الصناعية في المعمل قابلية للإصابة بهذين الفيروسين.

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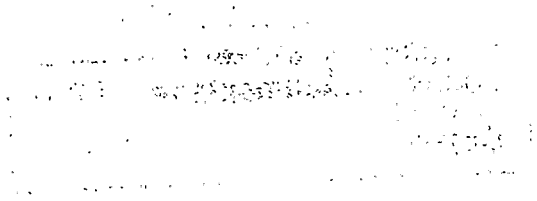

مطبوعة من

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مجلد ٢٤ العدد (١١)

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