Bull. Fac. Agric., Univ. Cairo, 47 (1996):179-190.

CHARACTERIZATION AND SEROLOGY OF THE LEAFHOPPER-BORNE MAIZE YELLOW STRIPE VIRUS IN EGYPT

(Received: 8.6.1995)

Jean-Claude

By

*J.C.Thouvenel, **S.Abol-Ela, **G.H.Sewify, ***M.El-Hariry, *A.Hussein and** E.D.Ammar

* ORSTOM, P.O.B 26, Giza.
** Dept. Economic Entomology, Faculty of Agriculture, Cairo University, Giza, Egypt.
*** Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt.

ABSTRACT

The nucleoprotein and non-capsid protein of maize yellow stripe virus (MYSV) were purified from naturally or experimentally infected maize or sorghum plants. In SDS-PAGE, the apparent molecular weight of the nucleoprotein was 35.6KD, and that of the non-capsid protein was 14.7KD. Similar to tenuiviruses, the nucleoprotein of MYSV was associated with fine filaments and the non-capsid protein formed typical needle-shaped crystals. Following a 2-day acquisition feeding period on MYSV-infected plants, vector leafhoppers *(Cicadulina chinai)* remained highly infective for 21 days.

Antisera to the nucleoprotein and non-capsid protein of MYSV were produced and used for detection of this virus in several host plants and vector leafhoppers in Egypt. Dot-blot and direct antigen coating (DAC) ELISA were used to detect MYSV in naturally or experimentally infected maize, wheat, barley, oat and the graminaceous weeds *Bromus wildenowii, Cenchrus biflorus, Dichanthium annulatum, Digitaria sanguinalis, Echinochloa colonum, Setaria viridis and S.verticillata.* Dot-blot and DAC-ELISA were used also to detect MYSV in naturally or experimentally infective leafhoppers.

Key words: *Cicadulina chinai* , Leafhopper vector, Maize yellow stripe virus, Tenuiviruses.

INTRODUCTION

In a survey of virus-like diseases in maize (*Zea mays* L.) during 1981 and 1982 in Egypt, a new disease designated maize yellow stripe virus (MYSV) was discovered (Ammar *et al.*, 1984). In 1985 and 1991 incidence of MYSV in certain parts of Egypt was very high particulary on Nili (Late-sown) maize plantations during August to October. (Ammar *et al.*, 1987, Abul-Ata, A.E., personal communication) . Ultrastructural studies revealed tubular helical structures in infected maize leaves showing MYSV symptoms; purified preparations from infected plants had typical nucleoprotein ultraviolet absorbance spectra, and contained tenuivirus-like filament 4-8nm in diameter. Additionally, similar to tenuiviruses, crystallized apparently non-capsid protein also was purified from infected plants (Ammar *et al.*, 1990b). However, unlike tenuiviruses, which are transmitted by the delphacid planthoppers (Gingerey, 1988), MYSV is persistently trasmitted by leafhopper *Cicadulina chinai* Ghauri, but not by *C. bipunctilla zea* China, or several other species of leafhoppers (Ammar *et al.*, 1984, 1989; 1990a). In ELISA tests, crude extracts from MYSV-infected leaves did not react with antisera to the capsid protein of maize stripe tenuivirus or to several other maize viruses (Ammar *et al.*, 1990b).

In the present work, the nucleoprotein and noncapsid protein of MYSV were purified and partially characterized using SDS-PAGE in addition to light and electron microscopy. Also we produced specific antisera to the nucleoprotein and noncapsid protein of MYSV; and we used these antisera, through ELISA and dot-blot methods, for detecting MYSV in several host plants and weeds as well as in the vector leafhopper *C.chinai*.

MATERIAL AND METHODS

Sources and maintenance of virus and vector:

The cultures of MYSV and *C.chinai* were the same as those used and refered to by Ammar *et al.*, (1989); MYSV was maintained in maize seedlings cv. SC10 or DC 204 in a greenhouse by serial transmission with *C. chinai*. Non-infective leafhoppers were maintained on caged healthy maize plants that were checked regularly for virus symptoms.

Purification of MYSV nucleoprotein:

The procedure used was modified after that reported by Ammar *et al.* (1990b). Infected maize or sorghum tissue (100-200g of leaves or stems) were blended in grinding buffer (1g/5 ml). After grinding for 3-5 min, the extract was filtered through cheesecloth and treated with 25% (v/v) chloroform After

shaking for 10 min, the emulsion was centrifuged at 7000rpm for 10 min. The clarified extract was centrifuged at 40 000 rpm for 2.5 hr at 5°C (Type 55.2 rotor). The pellets were resuspended in pellet buffer (0.01MKH2PO4, 0.01M Ethylene diamine tetraacetic acid (EDTA), pH 7.0) and kept overnight at 4°C or directly dissolved, stirred and were then layered onto 6ml of 20% sucrose (w/v) in pellet buffer and centrifuged at 4 000 rpm for 3 hr at 5°C. Pllets were resuspended in the pellet buffer and kept overnight at 4°C. The suspension was clarified and layered on 10-40% sucrose density grandients in phosphate buffer (0.01M KH2PO4, pH7.0) and centrifuged at 25 000 rpm for 4hr at 5°C (Beckman SW 28 rotor). One zone was observed by scanning at 260 nm wavelength, diluted in distilled water and pelleted at 40 000 rpm for 2 hr at 5°C then resuspended in phosphate buffer.

Virus buoyant density and electron microscopy:

Virus suspension was adjusted to 1.29g/ml Cs_2SO_4. After centrifugation for 16 hr at 38000 rpm at 5°C (Beckman SW 50 rotor), the gradient formed 1-2 bands that were collected using a syring needle.

For electron microscopy, grids were prepared using purified suspensions and contrasted with 5% uranyl acetate , or 2% PTA, and examined by transmission EM.

Purification of MYSV non-capsid protein:

Non-capsid protein was extracted from MYSV-infected tissue by the differential pH method (a modified method from that reported by Roca de Doyle *et al.*, 1992). Infected tissues (50g of leaves/stems) were ground in 100 ml of phosphate-citrate buffer (0.01MKH2PO4, 0.015M citric acid), pH 5.5. The extract was filtered through cheese cloth and the liquid kept overnight at 4°C to maximize crystallization of the protein. The extract was then centerifuged at 15000 g for 20 min.at 10°C. Pellets were resuspended in 50 ml of phosphate-citrate buffer, pH7.0, and the solution was clarified by centrifugation (15000g for 10 min) at 10°C. The supernatant was adjusted to pH 5.5 by adding 0.1 M citric acid, and again kept at 4 °C overnight to allow maximum recrystallization. Crystals were pelleted (15000g for 10 min) and the solubilization-recrystallization cycle was repeated five times or until the disappearance of the pellet, after clarification of the supernatant at pH7.0.

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE):

Capsid and non-capsid proteins were analysed by SDS-PAGE to estimate their molecular weight according to Laemmli (1970). Polyacrylamide

slab gels were composed of a 12% resolving gel and 5% stacking gel. Electrophoresis was at 136 V for 45min.

Persistence of MYSV in the vector:

Large numbers of healthy nymphs (4th-5th instar) or adults of *C.chinai* were fed on infected maize plants for a 2-day acquisition access period and transferred daily to healthy maize (10 insects/seedling) until all insects died. No. of indicator plants tested/day ranged from 12-29 plants.

Production and titration of antisera:

Two New Zealand rabbits were immunized by three weekly intramuscular injections of purified MYSV nucleoprotein emulsified with an equal volume of Freund's complete adjuvant for the first and second injections, and with incomplete adjuvant for the third injection. To prepare antisera of the non-capsid protein, incomplete adjuvant was used for the three weekly injections of this protein. In both cases, blood was taken on the 10th day after the third injection.

The titre of the produced antisera was determined by the agar double diffusion test; infected maize tissue was ground in 0.9% NaCl (1g/10ml) and squeezed throught two layers of cheese cloth. Nucleoprotein antiserum was cross absorbed against healthy maize extract, then mixed and centrifuged at 7000rpm for 10min. The pellet was discarded.

Enzyme-linked immunosorbent assay (ELISA) Test:

A direct antigen coating (DAC) ELISA was used as described by Convers and Martin (1990) with few modifications. Test samples were ground with a homogenizer at 1.10 dilution in coating buffer pH 9.6 and were applied to the microtiter plate and incubated overnight at 4°C. Wells were washed with PBS-Tween buffer 3 times, then a blocking agent (5% milk powder) was applied for 30 min at room temperature. Antiserum was then added at a dilution of 1/1000 in PBS-Tween buffer pH 7.4 containing 2% polyvinyl pyrolidone (PVP) and incuabted 3 hr at 37°C or overnight at 4°C. The plate was washed, dried and the conjugate to antirabbit alkaline phosphatase, diluted at 1/2000 in conjugate buffer containing ovalbumin 0.2g/l in PVP-PBS-Tween pH7.4 was added and incubated 3 hr at 37°C or overnight at 4°C The plate was then washed and dried. Paranitrophenyl phosphate (PNPP) was dissolved in substrate buffer pH 9.8 using 1mg/ml Absorbance values were measured at 405 nm wavelength. Absorbance values more than twice the value of healthy controls were considered positive

Dot-blot immunoassays of plants and leafhoppers:

The following method used was that of Peterschmitt, M. (personal communication). The nylon membrane (0.45 Um pore-Amersham) or nitrocellulose membrane (0.20 Um-Bio Bind-NC-Whatman) was directly used. Tissues (1g infected maize plant/10 µl buffer or 1 insect/10Ml buffer) were applied on this membrane after grinding in PBS buffer, pH 7.3 followed by clarification. The membrane was dried 5 min., immersed in PBS-Tween containing 5% milk, incubated for 45 min at 37°C and shaked for 15 min. It was then washed with PBS-Tween 3 times (2-5 min each) with shaking, incubated with antiserum at a dilution of 1/1000 in PBS-Tween for 2 hr at 37°C or overnight at 4°C, shaked for 15 min, then washed as above. The membrane was later incubated with conjugated anti-rabbit alkaline phosphatase diluted at 1/2000 in PBS-Tween for 2 hr at 37°C, then washed as above and incubated with substrate containing 30µ BCIP(5- Bromo-y-chloro-3-Indolyl phosphate) , 30µ NBT(Nitro Blue Terezolium)/15 ml substrate buffer, pH 9.8. The membrane was washed by distilled water to stop the reaction. All incubations were done in the dark.

RESULTS

Purification and characterization of nucleoprotein and non-capsid protein:

The extraction of MYSV from infected maize or sorghum tissue (Leaves or stems) was carried out using several buffers, (A) 0.2M KH2PO4, 0.001M diethyl dithiocarbamic acid (DIECA), 0.5% 2-mercaptoethanol, pH 8.0; (B) 0.01M KH2PO4, 0.01M EDTA, 0.5% 2- mercapthoethnol, pH 7.0; (C) 8g NaCl, 0.2g KH2PO4, 1.15g Na2HPO4, 0.2g KCL, 0.2 NaN3, pH7.4 that contained 0.05% Tween 20, 20g PVP; (D) 0.2 M Tris and 0.2 M boric acid, 20g PVP, 0.5% 2-mercaptoethanol, pH7.0. The highest concetration of purified virus was obtained using buffer (D). Concentration with high-speed centrifugation was better than the precipitation by polyethylene glycol (6% PEG, 0.2M NaCl) (Peterschmitt *et al.*, 1991). Virus obtained from leaves was about twice in concentration as high as that from stems, whereas little virus was obtained from roots. High concentration of purified virus was obtained from experimentally infected plants that were placed in a field plot covered with muslin, rather than from similar plants placed in cages in the greenhouse. The A 260/A280 ratio was 1.38 and 1.26 for field and greenhouse samples, respectively. Fine filaments 4-5 nm in diameter were detected by electron microscopy (after sucrose gradient centrifugation using 0.01 m KH2PO4, pH 7.0 or Cs_2 SO_4 isopycnic centrifugation using Tris buffer) following negative staining with uranyl acetate.

A non- capsid protein could be purified after several cycles of solubilization and crystallization. Crystallization was detected by the naked eye after one clarification spin in buffer at pH 7.0. citric acid was added to decrease the pH . No crystallization was obtained in similar preparations from healthy leaves. In SDS-PAGE protein released from purified nucleprotein preparations migrated as four protein bands of molecular weight 35.6 KD (major protein), 26.2, 21.7 and 13.6 KD (minor proteins) whereas the non-capsid protein migrated as 14.7KD (Fig1).

Persistence of MYSV in *C.chinai*:

Following a 2-day acquisition period, transmission rate of MYSV increased from 41% on day 7 to 90% on day 11 then continued between 60-95% up to 21 days post-acquisition (at 25°C temperature and 70% relative humidity) (Fig.2).

Serological tests on host plants:

MYSV was detected by DAC-ELISA or dot-blot in naturally infected maize and naturally infected graminaceous weeds: *Digitaria sanguinalis* and *Setaria virides*. Also, MYSV was detected serologically in experimentally infected maize, wheat, barley oat, and the graminaceous weeds: *Bromus wildenowii, Cenchrus biflorus, Dichanthium annulatum, Echinochloa colonum and S. verticillata.*

In DAC-ELISA, MYSV nucleoprotein antiserum had a titer of 10^{-5} with leaf extracts or purified virus diluted to 10^{-3} MYSV non-capsid protein antiserum was less sensitive (Fig. 3).

Serological tests on vector leafhoppers:

Using DAC-ELISA with MYSV nucleoprotein antiserum MYSV was detected in the clarified extracts of MYSV-infective *C.chinai* leafhoppers at a dilution of 10^{-2} (Fig.4).

Using dot-blot method, also with nucleoprotein antiserum, MYSV was detected in clarified extracts of single infective insects at a dilution of 1/4

DISCUSSIONS

In the present work MYSV nucleoprotein and non-capsid protein were purified and partially charecterized. Our results confirm the previous report (Ammar *et al.*,1990b), in which the nucleoprotein of MYSV was associated with tenuivirus-like filaments and the non-capsid protein formed

Fig. 1. SDS-polyacrylamide gel showing the single nucleoprotein (NP) non-capsid protein (NCP) of MYSV with purified healthy maize plants (H) and molecular weight markers (Ma).

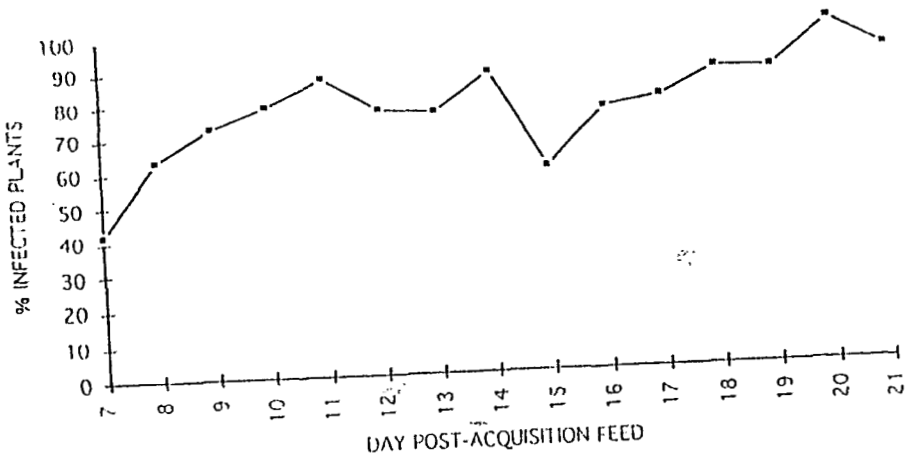
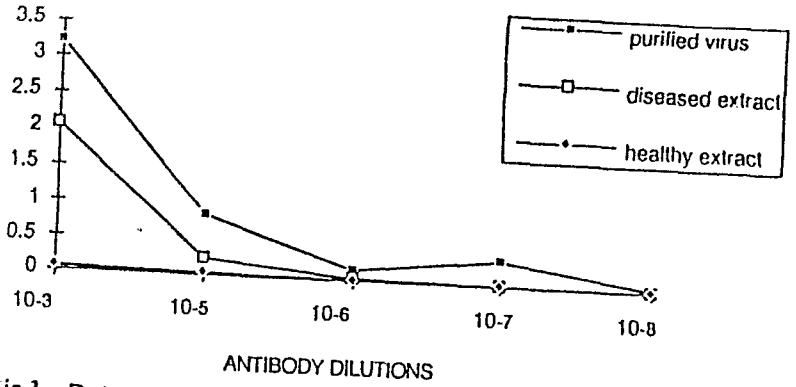
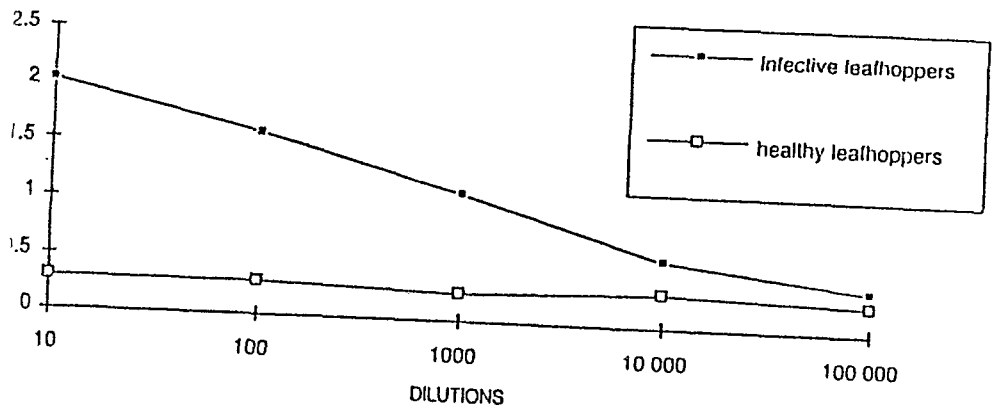


Fig.2. Percentage of successful transmission of MYSV by viruliferous *C. chinai* (10/maize plant) under greenhouse conditions (25°C temperature and 70% relative humidity). No. of indicator plants tested/day = 12-29 plants.



ig.3. Determination of antiserum titre using DAC-ELISA method

en was diluted to 10^{-3} in coating buffer. Antiserum was cross-absorbed against healthy maize t and incubated 3 hr/37°C. Conjugate was diluted 1/2000 in conjugate buffer and incubated ight/4 °C. Absorbence values were recorded after 24 hr.



.4. . Detection of MYSV in infective leafhoppers using DAC-ELISA.

)en dilution 10^{-1} in coating buffer. Antiserum was cross-absorbed 10^{-3} against healthy e extract and incubated in the plate 3.5 hr/37°C. Conjugate was diluted 1/2000 in conjugate r and incubated 4 hr/37°C. Absorbance values were recorded after 1 hr

crystals like those of previously described tenuiviruses (Gingery, 1988). Additionally, we estimated the molecular weight of MYSV nucleoprotein SDS-PAGE as 35.6 KD and that of the non-capsid protein as 14.7 KD. The three (minor) bands obtained in our SDS-PAGE of the nucleoprotein may have been caused by nucleoprotein degradation. The non-capsid protein of MYSV was estimated earlier as 13.8 KD by Ammar *et al.*, (1990b). Both estimates are slightly lower than those of non-capsid proteins of previously described tenuiviruses, whereas our estimate of the molecular weight of the MYSV nucleoprotein falls in the range of these viruses (Gingery, 1988).

Previous epidemiological studies on MYSV had to rely mainly on symptomatology and the laborious and time consuming insect-transmission tests for identification of this virus in different host plants in Egypt (Aboul-Ata and Ammar, 1985; Ammar *et al.*,1984, 1987, 1989; Sewify, 1994). Production and use of specific antisera for both the nucleoprotein and non-capsid protein of MYSV in the present work will facilitate further epidemiological studies on this virus and its vector in Egypt. Furthermore, it will help in the probable identification of this virus or serologically related viruses in other maize-producing countries particularly in Africa or the Middle East. The antiserum to the MYSV nucleoprotein seems more sensitive, and thus more useful in epidemiological studies, than that of the non-capsid protein. In our study, both DAC-ELISA and dot-blot methods were successful in detecting low concentrations of MYSV in host plants or in leafhopper vectors. DAC-ELISA is more economical and less complicated than other ELISA methods and thus is more useful in field surveys. However, the dot-blot method may be more sensitive than ELISA for detecting MYSV in single leafhoppers. Either method can be used for future studies to determine if MYSV replicates in its leafhopper vector, which is a crucial characterisatic for the classification of MYSV as a tenuivirus (Gingery, 1988; Ammar *et al.*, 1990b).

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توصيف وسيرولوجى لفيروس التخطط الأصفر فى الذرة الذى تنقله نطاطات الأوراق فى مصر

*جان كلود توفنيل ،** سعيد محمد أبوالعلا،** جمال حسن سويفى ، ***مجدى أحمد الحريرى ، *آمال حسين ، **الدسوقى أبو اليزيدعمار

* أورستم ص.ب. (٢٦) الجيزة

** قسم الحشرات الإقتصادية كلية الزراعة - جامعة القاهرة - القاهرة مصر

***معهد بحوث وقاية النبات ، مركز البحوث الزراعية الجيزة - مصر

ملخص

تمت فى هذا البحث تنقية و دراسة خواص كل من البروتين النووى غير الفيروسى لفيروس التخطط الأصفر فى الذرة من نباتات الذرة و السورجم المصابة بهذا الفيروس الذى تنقله نطاطات الأوراق نقلا إجباريا فى مصر. و قد كان الوزن الجزيئى للبروتين النووى ٣٥,٦ ك ٠.٥، و للبروتين غير الفيروسى ١٤,٧ ك د يكون البروتين النووى مصحوبا بخيوط دقيقة جدا مثل مجموعة فيروسات (Tenuiviruses) كما أن البروتين غير الفيروسى يكون بللورات بيرية . يبقى هذا الفيروس معديا فى الحشرات الناقلة (نطاط الأوراق *Cicadulina chinai*) لمدة ٢١ يوما بعد تغذية إكتساب قدرها يومان على النباتات المصابة.

هذا و قد تم إنتاج أنتيسيرم لكل من البروتين النووى و البروتين غير الفيروسى و يستخدم كل منهما فى إختبارات سيرولوجية (ELISA، Dot-Blot) للكشف عن هذا الفيروس فى الذرة و القمح و الشعير و الشوفان و بعض الحشائش النجيلية ، و كذلك للكشف عن الفيروس داخل الحشرات الناقلة له.

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (٤٧) العدد الأول (يناير ١٩٩٦):١٧٩-١٩٠.