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Occurrence of Lettuce Mosaic Virus in Alexandria and Effect of Infection on Seed Yield and Transmissibility

G.I.Fegla, Y.M.El-Fahaam, E.E.Wagih and **H.A.El-Karyoni**
Department of Plant Pathology, College of Agriculture,
University of Alexandria, Alexandria, Egypt

Abstract. Lettuce mosaic virus (LMV) was isolated from naturally infected lettuce plants and identified on the basis of symptomology, host range, cytopathology, physical properties in sap and serological reactions. The virus was designated as Alexandria isolate of LMV. The virus infected nine plant species belonging to 4 families and induced dense cytoplasmic amorphous inclusion bodies. In plant sap the virus had a thermal inactivation point between 50–60°C, a dilution end point between 10^{-2} –10^{-3}, longevity *in vitro* up to two days, and a positive serological reaction with LMV-specific antisera. The virus was seed-transmissible in the tested cultivars except for Vanguard–75 and Gallega cultivars. The percentage of seed transmission ranged from 2.3 to 5.1% in Paris Island and Green Boston respectively. As to local cultivars Eskandrany transmitted the virus at a relatively higher percentage (4.4%) than Balady (3.3%). Early infection of cultivars Balady and Eskandrany resulted in higher percentage of seed transmission and greater reduction in seed yield than late infection.

Introduction

Lettuce (*Lactuca sativa* L) has been shown to be infected with many diseases [1] of which the disease caused by lettuce mosaic virus (LMV) is of a special economic importance in all lettuce growing areas of the world [2,3,4].

Although the lettuce cultivated area in Egypt increased from 11,000 to 15,000 Feddans (Feddan = 4200 m^2) over a period of 6 years (1975 to 1981), little attention [5] has been given to LMV which is recognized as the most destructive agent to lettuce industry in the United States [1].

Mosaic symptoms similar to those caused by LMV were observed on lettuce plants grown at the College of Agriculture Experimental Farm of the University of Alexandria as well as at other different localities in Alexandria. Percentage of infected plants reached 100% by the head formation stage in most locations.

The present study was conducted to isolate and characterize the virus, to investigate its seed transmissibility and to determine the most important epidemiological factor associated with seed transmission in this disease.

Materials and Methods

Virus, plants and inoculation procedure

The virus was isolated from naturally infected lettuce (*Lactuca sativa* L. cv. Balady), grown at the Experimental Farm of the College of Agriculture, University of Alexandria and maintained in cultivar Balady or Eskandrany plants grown in insect proof cages. Infected leaf tissues were homogenized in a prechilled mortar and pestle, with 0.05 M phosphate buffer (pH 7.7) containing 0.1% 2– mercaptoethanol, using an extraction ratio of 1:4 (w/v). Inoculum was applied on selected host range and test plants previously dusted with carborundum (600 mesh) using a pad of cheesecloth, a cotton applicator or the index finger. These plants known to react characteristically with LMV [6,7,8], included *Chenopodium amaranticolor* Costa and Ryen, *C. quinoa* L., *Gomphrena globosa* L., and *Lactuca sativa L.*

Determination of physical properties in sap

The thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) of the virus were determined in sap extracted from infected cultivar Eskandrany lettuce plants, three weeks after inoculation. *Chenopodium amaranticolor* was used as a local lesion assay host [9, p. 207].

Serological tests

SDS-agarose double diffusion and chloroplast agglutination tests [9,10,11], were carried out using LMV antisera kindly provided by Drs. D.E.Purciful (Department of Plant Pathology, University of Florida, Gainsevill, USA) and L. Bos (Research Institute for Plant Protection, Wageningen, The Netherlands). The first test was conducted in Petri dishes containing 5 mm thick layer of agarose (0.8%) with 0.1% sodium azide and 0.5% sodium dodecyl sulfate. Infected lettuce leaves were first ground in distilled water (1 g/ml) and then 1 ml of 3% sodium dodecyl sulfate was added to the homogenate before straining through cheesecloth. Six peripheral and one central wells were punched in the agarose with a 5 mm d corkborer. The peripheral wells were filled with the treated plant extract while the specific antiserum was added to the central well. Treated plates were incubated for 2 days at 25°C in a moist chamber. The chloroplast agglutination test was performed using the standard technique described by Noordam [9]. Sap extracted from healthy plants was similarly treated for comparison.

Visualisation of inclusion bodies

Lower epidermal strips were removed by forceps from both virus infected and healthy leaves of cultivar Eskandrany and treated by two methods [9,12].

Seed transmission

a – Seed transmissibility tests

Seed transmissibility of the virus was tested by collecting seeds from infected lettuce cultivars Eskandrany and Balady inoculated at 7–10 leaf stage. Collected seeds were sown in pots and emerged plants showing mosaic symptoms were counted and percentage of seed transmission calculated (Seedling method). Similar lots of seeds were ground in the inoculum buffer and the resultant extracts were inoculated on *Chenopodium amaranticolor* leaves as described above. The number of local lesions per leaf was used as an estimate of the degree of seed transmission (Chenopodium method).

b – Lettuce cultivars

In order to determine seed transmissibility of LMV through different lettuce cultivars as well as to assess losses in seed yield as a result of infection, nine lettuce cultivars including two local (Balady and Eskandrany, Romaine type) and seven imported cultivars, Dark Green Boston (Buterhead type). Gallega (Latin type), Messa 659 (Crisphead type), Paris Island (Cos type), Salinas (Crisphead type), Vanguard–75 (Crisphead type), Waldman's Green (Leaf type) were used. The foreign cultivars were kindly supplied by Prof. E.J. Ryeder (Vegetable Production Research Unit, U.S.D.A., Agriculture Station, Salinas, California, USA), Prof. J.A.Tomlinson (National Vegetable Research Station, Wellesboren, Warwick CV 359 EF through the Vegetable Gene Bank, England, UK) and Prof. K.A.Kimble, (Department of Plant Pathology, University of California). Eighteen seedlings from each cultivar were transplanted individually, in 20 cm diam. pots and kept inside insect-proof cages until maturity and seed production. Half of these plants was inoculated, at 5–7 leaf stage, with LMV inoculum and the other half was mock-inoculated with phosphate buffer to serve as control. Treated plants were sprayed weekly with Malathion (1 ml/l) as an insecticide and Stemeful (0.5 g/l) as a foliar fertilizer. Seeds from each treatment were separately collected, weighed and assayed for virus transmission using both seedling method (growing on technique) and local lesion assay test on *Chenopodium amaranticolor*. Data obtained were statistically analyzed according to Snedecor [13, p. 534].

c – Age of lettuce plant at the time of inoculation

Thirty six lettuce seedlings from each of Balady and Eskandrany cultivars were transplanted individually in 20 cm. diam. Pots were divided into four groups, nine replicates each. The first group was left without inoculation to serve as control. The

second, third and fourth groups were inoculated with LMV inoculum at 5 leaf, 10 leaf and head forming stages, respectively. Plants were left till maturity and seed formation in insect-proof cages. Seeds of each treatment were separately collected, weighed and virus seed transmission was determined as previously outlined.

Results

Symptomology

Naturally infected lettuce plants showed typical mosaic symptoms accompanied by irregular growth of leaves and occasionally veinal necrosis and bronzing. Infected plants failed to form heads. Artificially inoculated plants developed symptoms identical with those found on naturally infected ones.

Diagnostic host reaction

Reactions on inoculated diagnostic host leaves indicated the presence of LMV. The virus induced pale green or chlorotic local lesions, usually with reddish margins on inoculated leaves of *C. amaranticolor* 8–10 days after inoculation, followed by systemic yellow veinal flecks 3–6 days later. Numerous local lesions but without reddish margins appeared on inoculated leaves of *C. quinoa,* 6–8 days after inoculation, followed by conspicuous systemic vein symptoms with twisting and stunting of apical leaves. *G. globosa* reacted, 4–7 days after inoculation, with whitish local necrotic dots enlarging into redrimmed spots. The virus induced systemic infection on lettuce (*Lactuca sativa* L.) and no reaction on *N. glutinosa.*

Physical properties in sap

The virus was found to have a TIP between 55–56°C, a DIP between 10^{-2} and 10^{-3} and LIV up to 2 days.

Serological reactions

In the chloroplast agglutination and SDS–agarose double diffusion tests, sap from infected Eskandrany plants reacted positively against LMV– specific antiserum. With the SDS – agarose technique a single precipitation band was observed between the infected lettuce sap and LMV–specific antisera, but no such a band was seen between healthy lettuce sap and the same antiserum.

Inclusion bodies

Inclusion bodies were detected within the cytoplasm of the leaf epidermal cells of LMV infected lettuce plants stained with either bromophenol blue [12] or rose bengal [9]. These inclusion bodies which appeared as dense amorphous bodies were usually located near the nucleus. No inclusion bodies were observed in leaf cells of healthy plants.

Host range

The virus could infect 9 plant species belonging to 4 families. The host range included *Carthumus tinctoris* L., *Chenopodium quinoa* L., *C. amaranticolor* Costa and Ryen, *C. murale* L., *Gomphrena globosa* L., *Lactuca sativa* L., cvs. Balady, Dark green Empire, Eskandrany, Great lakes 659, Royal, Salinas, Sea cream, Signal, Thompson and Valmine, *Malva parviflora, Pisum sativum,* L., *Spinacia oleracae.* In contrast, symptoms did not develop on, and the virus was not recovered from *Beta vulgaris, Brassica oleracea* var. capitata, *B. oleracea* var. botrytis, *Capsicum annum, Cucurbita maxima, Cucumis sativus, Datura stramonium, Glycine max* cvs. Bedford, Caland, Clark, Colombos, Crawford and Williams, *Helianthus annus, Lactuca sativa* L., cvs. Gallega, Serriola and Vanguard–75, *Lycopersicon esculentum, Nicotiana glutinosa, N. tabacum* vars. Turkish, White Buirly and Xanthi, *Phaseolus vulgaris* cvs. Swissblenth and Topcrop, *Vicia faba, Zea mays.*

Seed transmissibility of the virus

The virus was found to be transmissible through seeds of cultivars Eskandrany and Balady with the former being slightly more efficient (4.4%) than the latter (3.3%) in this respect (Table 1).

Table 1. Seed transmission of lettuce mosaic virus in lettuce cultivars Balady and Eskandrany

	Cultivar					
	Balady			Eskandrany		
Lot	Germinated* (A)	Infected (B)	% Infected (B/A × 100)	Germinated (A)	Infected (B)	% Infected (B/A × 100)
1	175	6	3.4	170	8	4.7
2	170	6	3.5	175	8	4.5
3	170	6	3.5	165	7	4.2
4	175	5	2.9	170	8	4.7
5	180	6	3.3	175	7	4.0
Mean	174	5.8	3.3	171	7.6	4.4

(*) Number of germinated seedlings out of 200 seed lots collected from LMV–infected plants.

The virus identified in this paper with the previous characteristics has been designed as LMV, Alex–isolate.

Some factors affecting seed transmissibility of LMV Alex. isolate

a – Lettuce cultivar

The 9 tested cultivars showed differences in seed transmissibility of LMV as evidenced by inspecting seedlings, produced from random samples of seeds, for LMV–

characteristic symptoms and by local lesion assay tests using *C. amaranticolor* (Table 2). Dark Green Boston gave the highest percentage of virus seed transmission (5.1%) followed by Salinas (4.7%) and Eskandrany (4.7%). The lowest percentage of virus transmission (2.3%) was observed in seeds of Paris Island. In contrast, seeds collected from csv. Gallega and Vanguard– 75 produced healthy seedlings.

Table 2. Seed transmission of LMV in lettuce cultivars

Cultivar	Seedling method		Chenopodium method	
	Germinated (A)[a]	Infected (A)	% Infected (B/A × 100)	No. of local lesion/leaf[b]
Balady	180	6	3.3	2
Dark green Boston	175	9	5.1	3
Eskandrany	170	8	4.7	3
Gallega	175	0	0.0	0
Mesa 659	175	5	2.9	2
Paris Island	175	4	2.3	2
Salinas	170	8	4.7	3
Vanguard–75	175	0	0.0	0
Waldman's Green	170	7	4.1	3

(a) Number of germinated seedlings out of 200 seed lots collected from LMV-infected plants.
(b) Inocula were prepared by grinding 200 seeds from each cultivar in 0.5 ml 0.05 M phosphate buffer, pH 7.7 and inoculated on 12 leaves distributed evenly on 3 *Chenopodium amaranticolor* plants.

Results presented in Table 3 showed that virus infection reduced seed yield. The percentage of reduction due to infection increased in the following order: Balady, Waldman's Green, Eskandrany, Salinas, Mesa of 659 and Paris Island. In contrast, Gallega and Vanguard–75 were the least sensitive cultivars.

b – Age of lettuce plant at the time of inoculation

The results presented in Table 4 revealed that the two cultivars, Balady and Eskandrany, carry the virus in their seeds regardless the tested time of inoculation with the second being more efficient than the first in this respect. This has clearly been demonstrated by both inspecting seedlings produced from 200 seed lots for LMV characteristic symptoms (seedling method) and by assaying virus content in similar lots of seeds using the local lesion assay test with *C. amaranticolor* as a local lesion reacting host (Chenopodium method). Additionally, inoculating plants with the virus at the early stages of growth (5 leaf and 10 leaf stages) resulted in higher percentage of seed transmissibility (2.30–5.88%) as compared to that obtained when

Table 3. Effect of infection with LMV on seed weight of nine lettuce cultivars

Cultivar	Average seed weight (g)*		
	Healthy (A)	LMV–infected (B)	% reduction $(\frac{A-B}{A} \times 100)$
Balady	0.26	0.19	26.9
Dark Green Boston	0.21	0.08	61.9
Eskandrany	0.24	0.14	41.7
Gallega	0.93	0.89	4.3
Mesa 659	0.18	0.10	44.4
Paris Island	0.15	0.08	46.7
Salinas	0.21	0.12	42.9
Vanguard–75	0.22	0.20	9.1
Waldman's Green	0.29	0.19	34.5

L.S.D.$_{0.05}$ among cultivars = 0.05

L.S.D.$_{0.05}$ between treatment = 0.02

* Figures are means of 9 replicates; each replicate represents the total yield of seeds produced from one plant.

plants were inoculated at the head stage (1.10–1.76%). The virus content in seed lots taken from plants inoculated at 5 leaf, 10 leaf and head stage decreased, as evidenced by the Chenopodium method, with the increase in the age of the plant at which inoculation was carried out (Table 4).

Table 4. Seed transmissibility of LMV in lettuce plants inoculated at different stages of growth

Cultivar	Time of inoculation (stage of growth)	Seedling method			Chenopodium method
		Germinated (A)[a]	Infected (B)	% infected (B/A × 100)	No. of local lesion/leaf[b]
Eskandrany	5–leaf	170	10	5.88	4.25
	10–leaf	170	6	3.50	3.25
	head	170	3	1.76	1.75
Balady	5–leaf	175	6	4.57	3.50
	10–leaf	175	4	2.30	2.75
	head	175	3	1.10	1.50

(a) Number of germinated seedlings out of 200 seed lots collected from LMV–infected plants.

(b) Inocula were prepared by grinding 200 seeds from each cultivar in 0.5 ml 0.05 M phosphate buffer, pH 7.7 and inoculated on 12 leaves distributed evenly on 3 *Chenopodium amaranticola* plants.

Figures are significantly different at a probability level of 0.05; each test was repeated three times.

Inoculation of Balady and Eskandrany cultivars at any stage of growth with LMV has affected seed weight. Cultivar Eskandrany was more sensitive to infection than Balady cultivar. The reduction in seed weight was greater when infection occurred at early stages of plant development (Table 5).

Table 5. Seed yield of Eskandrany and Balady lettuce cultivars inoculated with LMV at different stages of growth.

Time of inoculation	Cultivars			
	Eskandrany		Balady	
	Seed weight		Seed weight	
	in g.	% reduction	in g.	% reduction
Control	0.22	–	0.27	–
5-leaf stage	0.02	90.9	0.16	40.7
10-leaf stage	0.03	86.4	0.19	29.6
Head stage	0.08	63.4	0.27	00.0
L.S.D$_{0.05}$ among stages = 0.04				

Data are average of 9 replicates.

Discussion

On the basis of symptomology, reactions on diagnostic hosts serological properties, host range studies, cytopathological effects, physical properties in sap, and seed transmissibility, the virus isolated in this study was identified as lettuce mosaic virus (LMV). The isolated virus was designated as LMV, Alex – isolate.

The symptoms observed on naturally or artificially infected lettuce plants as well as on inoculated diagnostic hosts were similar to those reported for LMV [7,8,14,15, p. 648].

The positive serological reaction observed with two preparations of LMV – antisera obtained from different sources confirmed that the isolated virus is LMV.

The results of host range studies were in accordance with those reported for LMV in earlier studies [14,16,17,8].

The obtained physical properties of the virus in sap are like those previously reported for LMV by several authors [5,15,17,8].

The amorphous granular inclusion bodies detected in the lower epidermal strips of diseased lettuce leaves are typical to those shown by Shawkat *et al.* [17] in LMV-infected lettuce. The experiments conducted herein revealed that LMV can be trans-

mitted through seeds of infected lettuce plants. This finding is in agreement with those reported by Grogan *et al.*[18].

The variation observed in the current work among lettuce varieties with respect to virus seed transmission was also reported with different isolates by other investigators. Fegla *et al.* [19] working with an Iraqi isolate of LMV found that lettuce cv. White Boston infected at different stages of development produced seeds with a higher percentage of virus transmission in comparison to other cultivars, while the cultivar Paris Green infected at 5–7 leaf stage or head formation stage had a lower percentage of infected seeds.

Percentage of virus seed transmission was also found to be affected with the time of inoculation. Our results indicated that infection of lettuce cvs. Balady and Eskandrany at early stages of plant development (5 or 10 – leaf stage) resulted in higher percentage of seed transmission than infection at later (head formation) stage. The same conclusion was reached by Couch [20] and Fegla *et al.* [19] using different cultivars.

The weight of seed yield was also affected by LMV–infection. The effect was greater when infection occurred at early stage of plant development. Such results coincide with those reported by Fegla *et al.* [19] and Ryder and Duffs [21].

The data presented in this work show that LMV is prevalent in Alexandria lettuce plantations. The fact that resistant cultivars such as Vanguard–75 and Gallega do not carry the virus in their seeds may suggest the use of such cultivars in Egypt to eliminate the primary source of inoculum. However, the suitability of Egypt environment to the growth and productivity of these cultivars remains to be seen. If susceptible cultivars are thought after, the use of virus-free seeds could be another alternative to control the disease.

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وجـود فيروس موزايـك الخس في الإسكندرية وتأثير العدوى على محصول البذرة والقابلية للنقل بها

جابر أحمد إبراهيم فجله، يحيى محمد حامد الفحام، السيد السيد وجيه وحافظ أحمد الكريوني

قسم أمراض النبات، كلية الزراعة، جامعة الإسكندرية، الإسكندرية، جمهورية مصر العربية

ملخص البحث. تم عزل فيروس موزايك الخس من نباتات خس مصابة طبيعيًّا وتم تعريف الفيروس على أساس الأعراض والمدى العائلي والتغيرات المرضية الخلوية والصفات الطبيعية للفيروس في عصارة النبات والتفاعلات السيرولوجية. وقد أطلق على الفيروس عزلة الإسكندرية.

أوضحت الدراسة أن الفيروس يصيب تسعة أنواع نباتية تتبع أربع عائلات ويدفع الخلايا المصابة لتكوين أجسام محتواه أميبية في السيتوبلازم. والفيروس في عصارة النبات كان له نقطة تثبيط حراري بين ٥٠-٦٠°م ونقطة تخفيف نهائية بين $١٠^{-٢}$ - $١٠^{-٣}$ ومدة بقاء خارج الخلية حتى يومين. وقد أعطى تفاعل سيرولوجي موجب مع أمصال مضادة متخصصة لفيروس موزايك الخس. وقد ثبت أن الفيروس ينتقل بالبـذور في جميع الأصناف التي اختبرت فيما عدا الصنفين فان جارد - ٧٥ (Vanguard - 75) وجاليجا (Gallega). وقـد تراوحت النسبـة المئوية للنقل بالبذرة من ٢ر٣ إلى ١ر٥٪ في الصنف باريس أيلاند (Paris Island) والصنف جرين بوستون (Green Boston) على الترتيب. وفيما يختص بالأصناف المحلية فالصنف اسكندراني نقل الفيروس عن طريق البذور بنسبة مئوية أعلى نسبيًّا (٤ر٤٪) من الصنف بلدى (٣ر٣٪) كما أوضحت الدراسة أن العدوى المبكرة للصنفين بلدى وإسكندراني تسببت في زيادة النسبة المئوية للنقل بالبذرة وانخفاض أكبر في محصول البذور عن العدوى المتأخرة.