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Lettuce Varietal Response and Possible Mechanism of Resistance in Lettuce to Lettuce Mosaic Virus

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Abstract. Twelve lettuce cultivars including 2 local (Balady and Eskandrany) and 10 imported were evaluated for resistance to lettuce mosaic virus (LMV). All tested cultivars, except Vanguard-75 were susceptible, with different degrees. Cultivars Balady, Empire, Sea Cream, and Signal showed the least percentage of infected plants (40-50%) as compared to Eskandrany, Great Lakes 659 and Thompson (60-70%). The most susceptible were Dark Green, Royal, Salinas, and Valmine with a percentage of infection ranging from 80-100%.

Symptoms in Balady and Eskandrany appeared in 8 to 10 days after inoculation with LMV or LMV-RNA and virus replicated well in these cultivars. Extract of Vanguard-75 when tested in vitro inhibited the infectivity of LMV-infected Eskandrany inocula. The mechansim of resistance in Vangaurd-75 may probably be due to failure of accomplishment of some early events of infection or replication.

Introduction

Lettuce (*Lactuca sativa* L.) is susceptible to several viruses in nature among which lettuce mosaic virus (LMV) is the most destructive [1]. Since the disease was first reported [2] a volumenous work has been going on studying the disease and finding a suitable way of controlling it. Selection and/or breeding for resistance to LMV seems to be the most effective method so far in controlling the disease. Germplasms resistant to LMV were found in wild lettuce [3] as well as in commercial cultivars [4,5]. Efforts to incorporate genes for resistance from these sources into commercial cultivars has been successful [6]. However, the mechanism of resistance to LMV has always been a matter of speculation.

The fact that lettuce mosaic virus is a widespread disease affecting lettuce plants in Egypt [7] has tempted us to evaluate some local as well as foreign lettuce cultivars

for resistance to LMV and to investigate the host-virus interaction to elucidate the probable mechanism of resistance operating in LMV resistant cultivars.

Materials and Methods

Virus and inoculation technique

The lettuce mosaic virus isolate (LMV-Alex.) previously identified by Fegla *et al.* [7] was used. Inocula were prepared from systemically infected lettuce cv. Eskandrany leaves showing mosaic symptoms. Infected tissue was homogenized in 0.05 M phosphate buffer, pH 7.7 (1:4, m/v) containing 0.1% 2-metoaptoethanol using an ice cold mortar and pestle.

Plants were inoculated with either the whole virus or its RNA using a pad of cheesecloth with carborundum (600 mesh) as an abbrasive. Control plants were mock-inoculated with phosphate buffer. All tests plants were kept in insect-proof greenhouses at 22 ± 2°C.

Preparation of viral-RNA

Lettuce mosaic virus ribonucleic acid (LMV-RNA) was extracted from infected Eskandrany lettuce leaves following the method adopted by Jackson *et al.* [8] with slight modification. Frozen tissue (0.2 g) was homogenized in a mortar and pestle at 4°C with 1 ml TNE buffer (0.1 M Tris-HCl, 0.1 M NaCl, 0.01 M Na_2 EDTA, pH 8.5, 2% SDS) added together with an equal volume of a mixture of water saturated phenol (8.5 vol.), m-cresol (1.5 vol.) containing 0.1% P-hydrox quinoline and 0.025 ml diethylpyrocarbonate. The mixture was shaken at 4°C for 10 min. The aqueous phase, recovered by centrifugation at 3000 g for 10 min., was re-extted with phenol and reseparated. The remanant phenol was removed by several washings with diethylether and the residual ether evaporated with an air stream.

Evaluation of lettuce cultivars for resistance to LMV

Ten plants from each cultivar at 5 leaf stage were mechanically inoculated with LMV as described above. The same number of plants at the same age were inoculated with the buffer to serve as control. Virus and buffer inoculated plants were kept in insect-proof cages. Disease incidence and severity of symptoms were recorded 3 weeks after inoculation. The experiment were repeated twice.

Studying the mechanism of resistance in Vanguard-75

Twenty plants from each of the two cultivars Eskandrany and Vanguard-75 were inoculated with either the whole virus, nucleoprotein (LMV), or its nucleic acid (LMV-RNA). Inoculated plants were kept in an insect-proof cage and observed for

the development of symptoms. Leaf samples were collected at 0, 5, 10, 15 and 30 days after inoculation and ground in phosphate buffer (1 g/l ml). The homogenate obtained from each sample was inoculated onto three plants of *C. amaranticolor* Costa and Ryen, each with 4 leaves. Two weeks after inoculation, the average number of local lesions per leaf was calculated. Data were first transformed with $\sqrt{x+1}$ before statistical analysis.

Testing the possible inhibitory effect of lettuce sap on LMV infectivity

To determine the possible inhibitory effect of resistant lettuce cultivar (Vanguard-75) on virus multiplication, inocula from infected Eskandrany, healthy Eskandrany and health Vanguard-75 were prepared in the phosphate buffer (1:4, m/v). Infective sap was diluted with healthy Vanguard-75 leaf extracts in various ratios (4:0, 3:1, 2:2, 1:3, 0:4). Similar preparations of infective Eskandrany sap diluted with healthy Eskandrany sap were used as control. All preparations were incubated for 1 h at room temperature (25 ± 2°C). Each preparation was then separately inoculated onto the leaves of three *C. amaranticolor* plants with 4-leaves each. Two weeks after inoculation the average number of local lesions per leaf was calculated and data were statistically analyzed.

Results and Discussion

Evaluation of lettuce cultivars for resistance to LMV

Twelve lettuce cultivars including 2 local and 10 imported cultivars representing three types, Crisphead, Cos, and Romaine were evaluated for their resistance to LMV and found to differ in their reaction. The results presented in Table 1 show that all the cultivars except Vanguard-75 were more or less susceptible to LMV. Balady, Empire, Sea cream, and Signal cultivars showed the least percentage of infected plants (40-50%) as compared to Eskandrany, Great Lack 659, and Thompson which exhibited relatively higher values (60-70%). The most susceptible cultivars were Dark Green, Royal, Salinas and Valmine with a percentage of infection ranging from 80-100%.

In Vanguard-75, resistance was demonstrated by the lack of mosaic symptoms and the inability to recover the virus from inoculated plants. Although the results presented here disagree with those reported by Ryder [6,9], the finding that the escape ability of the genetically resistant plants is a function of either the environment or the plants themselves at the time of inoculation and afterwards [6] may account for this discrepancy. In spite of the fact that the same single recessive gene (mo) confers resistance in all LMV resistant cultivars [4,6], the results obtained by Walkey *et al.* [5] indicated that the protection given by the (mo) gene is affected by environment conditions.

Table 1. Evaluation of lettuce cultivars for resistance to lettuce mosaic virus

Cultivar*	Type	% infected plants	Symptom severity
Balady	Romine	40	V + mM
Dark Green	Cos	80	V + mM
Empire	Crisphead	40	V + mM
Eskandrany	Romine	70	V + M
Great Lakes 659	Crisphead	60	V + mM
Royal	Romine	90	V + M
Salinas	Crisphead	100	V + SM
Sea cream	Crisphead	50	V + mM
Signal	Cos	50	V + mM
Thompson	Crisphead	70	V + mM
Valmine	Cos	90	V + mM
Vanguard-75	Crisphead	0	–

V = Vein clearing; mM = Mild mosaic symptoms; M = Moderate mosaic symptoms; SM = Sever mosaic symptoms; – = No symptoms were observed; * = Ten plants from each cultivar were inoculated at the 5 leaf stage and the test was repeated twice.

Studying the mechanism of resistance in Vanguard-75

Lettuce mosaic virus (whole virus) and its nucleic acid (LMV-RNA) preparations were tested for their infectivity on Vanguard-75 (Resistant), Eskandrany and Balady (susceptible) cultivars. It was observed that both preparations could not infect Vanguard-75. However, Eskandrany as well as Balady could be infected with either LMV-whole particle or LMV-RNA. In both susceptible cultivars, symptoms appeared 8 days after inoculation with LMV-RNA but took 10 days when LMV-whole particles were used as an inoculum.

The inoculum from Eskandrany samples, 0, 5, 10, 15 and 30 days after inoculation with the whole virus produced an average number of local lesions of 1.58, 0.41, 2.61, 1.83/leaf on *C. amaranticolor*. No infectivity was recovered from any of the inoculated Vanguard-75 samples. This demonstrates that virus did multiply in susceptible plants but in resistant cultivar no multiplication occured. With LMV-RNA, virus replication increased with time after inoculation of plants and reached to maxima (2.5 local lesion/leaf) in 15 days.

The ability of LMV-RNA to infect Eskandrany and Balady cultivars but not Vanguard-75 may suggest that the mechanism of resistance is not due to a failure in the process of virus coat protein-host cell interaction but due probably to a cytoplasmic factor that is present in the resistant but not in the susceptible cultivars. This

hypothesis is shown to be highly likely by the ability of healthy Vanguard-75 leaf extracts to reduce the infectivity of LMV-infected Eskandrany leaf extracts in a concentration dependent manner.

Inhibitory effect of healthy Vanguard-75 extracts on LMV infectivity

Buffered extracts from healthy Vanguard-75 caused significant reduction in the infectivity of LMV in Eskandrany extracts when mixed with it at ratios of 1:1 or 3:1 but not at lower ratios (i.e. 1:3 or 0:4). Comparisons were always made with LMV-infected Eskandrany extracts mixed with corresponding amounts of healthy Eskandrany extracts to those used from the healthy Vanguard-75 extracts (Table 2).

Table 2. The inhibitory effect of healthy Vanguard-75 extracts on the infectivity of LMV-infected Eskandrany leaf extracts.

Treatment	No. of local lesions per leaf*	
	H. Vg-75	H.Ek.
4 ml Inf. Esk. + 0 ml H. Vg-75 or H. Esk	2.00	2.00
3 ml Inf. Esk. + 1 ml H. Vg-75 or H. Esk	1.75	1.50
2 ml Inf. Esk. + 2 ml H. Vg-75 or H. Esk	1.58	2.25
1 ml Inf. Esk. + 3 ml H. Vg-75 or H. Esk	0.75	2.58
0 ml Inf. Esk. + 4 ml H. Vg-75 or H. Esk	0.00	0.00

$L.S.D_{0.05}$ between mixtures = 0.4

* Average number of local lesions per leaf, calculated from lesions developed on 12 leaves on 3 *C. amaranticolor* plants each with 4 leaves.

H = Health, Inf. = Infected, Esk. = Eskandrany, Vg-75 = Vanguard-75.

The inhibitory effect of the healthy Vanguard-75 extract on the infectivity of LMV-infected Eskandrany cultivar may have been due to the presence of an inhibitory chemical substance(s) in the Vanguard-75 extracts. This suspected chemical substance(s) may have acted by altering host cell metabolism so that cells are no longer susceptible to the virus [10-14]. Alternatively, this substance(s) could have directly interferred with virus replication at the cytoplasmic level.

Once this substance is chemically identified, it could be synthesized in the laboratory and used for controlling the disease in the field. Another approach is to introduce the gene(s) coding for this chemical substance into susceptible lettuce cultivars using the modern techniques of Genetic Engineering, but this depends totally on the mechanism involved.

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References

[1] Grogan, R.G. "Control of Lettuce Mosaic with Virus-Free Seed". *Plant Dis.*, **64** (1980), 446-449.

[2] Jagger, I.C. "A Transmissible Disease of Lettuce". *J. Agric. Res.*, **20** (1921), 740.

[3] Ryder, E.J. "Evaluation of Lettuce Varieties and Breeding Lines for Resistance to Common Lettuce Mosaic Virus". *USDA Tech. Bull.*, **1391** (1968), 8.

[4] Ryder, E.J. "Inheritance of Resistance to Common Lettuce Mosaic". *J. Amer. Soc. Hort. Sci.*, **95** (1970), 378-379.

[5] Walkey, D.G.A., Ward, C.M. and Phelips, K. "Studies on Lettuce Mosaic Virus in Commercial Lettuce Cultivars". *Plant Pathol.*, **34** (1985), 535-551.

[6] Ryder, E.J. "The Nature of Resistance to Lettuce Mosaic". *Eucarpia Meeting on Leafy Vegetables Wageningen - Holland,* 15-18 Mash, (1976), 110-118.

[7] Fegla, G.I., El-Fahaam, Y.M., Wagih, E.E. and El-Karyoni, H.A. "Occurrence of Lettuce Mosaic Virus in Alexandria and Effect of Infection on Seed Yield and Transmissibility". *J. King Saud Univ. Agric. Sci.*, **2** No. 1 (1990).

[8] Jackson, A.O., Raitlin, M., Siegel, A., and Franckl, R.I.B. "Replication of Tobacco Mosaic Virus 111. Viral RNA Metabolism in Separated Leaf Cells". *Virology,* **48** (1972), 655-665.

[9] Ryder, E.J., "Salinas" Lettuce 1." *Hort. Science,* **14** (1979), 283-284.

[10] Bawden, F.C. and Freeman, G.G. "The Nature and Behaviour of Inhibitor of Plant Viruses Produced by *Trichothecium roseum* Link." *J. Gen. Microbiol.*, **7** (1952), 154-168.

[11] Bawden, F.C. "Physiology of Virus Disease." *Rev. Plant Physiol.*, **10** (1954), 239-256.

[12] El-Kandelgy, S.M. and Wilcoxon, R.D. "Effect of Red Clover Extract and Glucose on Infection of *Gomphrena globosa* by Red Clover Vein Mosaic Virus." *Phytopathology,* **56** (1966), 832-837.

[13] McKeen, C.D. The Inhibitory Activity of Extracts of *Capsicum frutescens* on Plant Virus Infections." *Can. J. Bot.*, **34** (1956), 891-903.

[14] Simons, I.N., Widler, R. and Moss, L.M. "Succulent Type Plants as Source of Plant Virus Inhibitors." *Phytopathology,* **53** (1963), 677-683).

استجابة أصناف الخس وميكانيكية المقاومة المحتملة في الخس لفيروس موزايك الخس

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ملخص البحث. تم تقويم ١٢ صنفًا من الخس، إثنين محليين (بلدي وإسكندراني) وعشرة مستوردة، للمقاومة لفيروس موزايك الخس. وقد اتضح أن كلّ الأصناف المختبرة فيما عدا فان جارد ـ ٧٥ (Vanguard - 75) كانت قابلة للاصابة بدرجات مختلفة. وقد أظهرت الأصناف بلدى، إمبير (Empire) وسى كريم (Sea Cream) وسيجنال (Signal) أقل نسبة من النباتات المصابة (٤٠ـ٥٠٪) بالمقارنة بالأصناف إسكندراني والجريت ليكس ٦٥٩ (Great lakes 659) وتومسون (Thompson) (٦٠ـ٧٠٪) وكانت أكثر الأصناف قابلية للإصابة دارك جرين (Dark Green) ورويال ساليناس (Royal Salinas) وفالماين (Valmine) بنسبة إصابة مئوية تتراوح بين ٨٠ـ١٠٠٪.

وقد ظهرت أعراض الإصابة على الصنفين بلدى وإسكندراني في ٨ـ١٠ أيام بعد العدوى بفيروس موزايك الخس أو بحامضه النووي ريبونيوكلييك أسد (RNA) وتكاثر الفيروس جيدًا في هذين الصنفين ـ هذا وعندما اختبر مستخلص الصنف فان جارد ـ ٧٥ خارج الخلية وجد أنه قادر على تثبيط قدرة اللقاحات المحضرة من الصنف إسكندراني المصاب بفيروس موزايك الخس، ويحتمل أن تكون ميكانيكية المقاومة في الصنف فان جارد ـ ٧٥ راجعة إلى فشل اكتمال بعض الأحداث المبكرة في عملية العدوى أو تكاثر الفيروس.