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Thermotherapy of Lettuce Mosaic Virus Infected Seeds

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

Abstract. Treating lettuce mosaic virus (LMV) infected seeds by dry heat at 40, 50, 60, 70, 80 and 85°C for 1, 2, 3, and 4 days showed that exposure to 85°C for any of the tested periods eradicated the virus, but greatly reduced seed germination. However, treating seeds at 80°C for 3 or 4 days was effective in eradicating the virus without a significant reduction in seed germination. Imbibing LMV-infected seeds in polyethylene glycol (PEG) and heating at 22, 38 and 40°C for 5,10 and 15 days resulted in complete inactivation of the virus in both cultivars, Balady and Eskandrany, with no significant effect on seed germination only when seeds were imbibed in PEG and heated at 40°C for 10 days.

Introduction

Lettuce mosaic virus (LMV) is a serious pathogen to lettuce (*Lactuca sativa* L.). The virus is a member of the potyvirus group [1] easily transmitted by mechanical means [2] and is also seed transmissible [3] at a ratio of 1-10% [4-6]. Infected seedlings produced from few seed-borne infections provide a primary source of inoculum from which virus spreads mainly by aphids in a non-persistent manner [7].

The use of LMV-free lettuce seeds for controlling LMV has previously been suggested [3,6] and would be extremely useful to farmers. This work was thus undertaken to determine if LMV carried in infected seeds could be eradicated by dry and/or wet heat treatment.

Materials and Methods

Virus and inoculation procedure

A previously identified isolate of lettuce mosaic virus (LMV, Alex-isolate) [8] was used in this study. Inocula were prepared and test plants inoculated as previously

outlined [8]. Control plants were treated with buffer and abrasive. After inoculation all plants were placed inside insect-proof cages.

Heat treatment

Seeds collected from infected lettuce plants of the cultivars "Balady" and "Eskandrany" inoculated with LMV at 7-10 leaf stage and with a known percentage of LMV-infected seeds, as evidenced by symptoms produced on seedlings, were used in the following heat treatments.

A) Dry heat treatment

Samples of 200 seeds each were placed in paper bags and placed in a dry air oven at 40, 50, 60, 70, 80 or 85°C for a period of 1, 2, 3 or 4 days. Three replicates were used for each treatment. Seeds used as control were kept at room temperature. Seed germination and seedling infection rates were recorded and statistically analysed [9, p. 327].

B) Wet heat treatment in the presence of polyethylene glycol

Polyethylene glycol (PEG, MW 6000) dissolved in distilled water at the concentrations of 360, 406 and 415 g/l was used to give nominal 15 bar osmotic potential at 22, 38 and 40°C, respectively. Eight ml of the PEG solution were added to 9 cm diameter Petri dish containing four layers of Whatman No. 1 filter paper and samples of 200 seeds each were placed onto the moist paper. Dishes were sealed with parafilm, placed in polyethylene bags, and incubated at 22, 38 or 40±1°C for 5, 10 or 15 days. Similar samples, without PEG, were similarly treated as control. After heat treatment seeds were removed from the Petri dishes and rinsed with 100 ml of 2% sodium hypochlorite solution to eliminate microbial contamination. Surface sterilized seeds were then rinsed twice with 100 ml distilled water to remove the residual PEG and sodium hypochlorite. Seed germination and seedling infection rates were recorded and statistically analyzed as previously mentioned.

Results

Inactivation of LMV in lettuce seeds

A) Dry heat treatment

Treating LMV-infected seeds of lettuce cultivars Eskandrany and Balady with dry heat at 40, 50, 60, 70, 80 or 85°C for 1,2,3 or 4 days has shown that exposure to 85°C for any of the tested periods eradicated the virus but greatly reduced percentage of germination (Table 1 and 2). Treatment with 80°C, however, was effective in eradicating the virus only when seeds were exposed to this temperature for 3 or 4 days but not for shorter periods. At this temperature, germination percentage

Table 1. Effect of hot air on seed transmission of lettuce mosaic virus in Eskandrany lettuce seeds

Temp-rature (°C)	Exposure period (days)	Seed germination		Seed transmission		Temperature means		Exposure period		Treatment means	
		%	Trans.[a]	%	Trans.[b]	a	b	a	b	a	b
	1	99.6	87.8	7.1	15.7						
	2	91.1	85.6	7.4	15.8						
40	3	98.0	88.4	7.6	16.3	85.1	16.0				
	4	96.1	78.8	7.8	16.2						
	1	98.7	84.8	8.2	16.6						
	2	96.5	79.2	7.2	15.6						
50	3	95.2	77.6	6.8	15.1	78.9	15.6				
	4	92.4	74.0	6.9	15.2						
	1	96.7	79.8	7.0	15.3						
	2	95.2	77.4	5.7	14.0	81.3	14.0	71.6	11.2		
60	3	92.8	74.5	5.9	14.0			69.7	10.7		
	4	91.7	93.3	5.0	12.8			67.2	10.0	69.3	10.3
								68.7	9.2		
	1	91.1	72.6	7.1	15.1						
	2	88.6	72.6	5.1	13.1	72.9	12.8				
70	3	88.4	70.3	5.2	13.0						
	4	87.8	76.2	2.9	9.1						
	1	91.9	73.5	0.5	3.4						
	2	91.1	72.8	0.1	4.8						
80	3	87.3	69.2	0.0	0.7	70.1	2.4				
	4	81.7	64.7	0.0	0.7						
	1	26.6	31.0	0.0	0.7						
	2	25.7	30.5	0.0	0.7						
85	3	22.6	28.4	0.0	0.7	28.7	0.7				
	4	17.9	24.9	0.0	0.7						
Control at room tempera-ture	1	99.8	84.4	7.4	15.7						
	2	100.0	90.0	8.3	16.7						
	3	99.8	88.4	8.1	16.5	87.4	16.4				
	4	99.5	86.8	8.1	16.5						

$L.S.D_{0.05}$	Seed germination	Seed transmission
Temperature	2.1	0.9
Exposure period	1.6	0.7

Trans.[a] and trans.[b] are the transformed values corresponding to seed germination and virus seed transmission percentages, respectively. To allow statistical analysis, data as percentages were first transformed as described by Snedecor [9, P. 327]. Data are average of 3 replicates.

Table 2. Effect of hot air on seed transmission of lettuce mosaic virus in Balady lettuce seeds

Temp-rature (°C)	Exposure period (days)	Seed germination		Seed transmission		Temperature means		Exposure period		Treatment means	
		%	Trans.[a]	%	Trans.[b]	a	b	a	b	a	b
	1	99.7	84.7	7.2	15.6						
	2	95.3	78.0	7.3	15.7						
40	3	92.9	81.0	6.8	15.2	78.7	15.4				
	4	89.5	71.2	6.9	15.2						
	1	96.0	77.2	6.6	16.0						
	2	94.4	76.4	6.3	14.5						
50	3	92.4	74.0	5.8	13.9	74.8	13.6				
	4	90.1	71.7	3.8	11.3						
	1	92.4	75.1	6.4	14.7						
	2	91.0	72.5	6.2	14.5						
60	3	89.1	70.4	3.9	11.3	71.8	12.5				
	4	87.1	69.0	2.8	9.6			66.4	10.6		
								64.1	10.1	63.4	9.4
	1	86.7	68.6	5.6	13.7			63.3	9.1		
	2	85.1	67.3	5.0	12.6	66.3	11.8	59.6	7.6		
70	3	82.2	65.1	4.7	12.5						
	4	80.7	64.0	2.0	8.2						
	1	85.5	67.6	0.7	4.1						
	2	83.1	65.7	0.7	4.1						
80	3	82.6	65.4	0.0	0.7	65.0	2.4				
	4	77.1	61.4	0.0	0.7						
	1	18.0	25.1	0.0	0.7						
	2	17.2	24.5	0.0	0.7						
85	3	16.2	23.6	0.0	0.7	23.3	0.7				
	4	14.1	20.0	0.0	0.7						
Control at room tempera-ture	1	100.0	90.0	8.1	16.5						
	2	100.0	90.0	6.7	15.0						
	3	99.5	86.8	7.3	14.7	88.4	15.5				
	4	99.5	86.6	7.6	15.9						

$L.S.D_{0.05}$	Seed germination	Seed transmission
Temperature	2.1	0.8
Exposure period	1.6	0.6

Trans.[a] and trans.[b] are the transformed values corresponding to seed germination and virus seed transmission percentages, respectively. To allow statistical analysis, data as percentages were first transformed as described by Snedecor [9, P. 327]. Data are average of 3 replicates.

remained at a relatively high level (87.3 - 81.7 in Eskandrany and 82.6-77.1 in Balady).

B) Wet heat treatment with polyethylene glycol

Results presented in Tables 3 and 4 indicate that, in both cultivars, imbibing seeds in PEG and heat treating at 22, 38 or 40°C for 5 days did not free seeds from the virus. In contrast, when seeds were imbibed in PEG and heated at 40°C for 10 days complete inactivation of the virus in both cultivars was achieved with no effect on germination. Although imbibing seeds in PEG and heat treating at 40°C for 15 days could similarly inactivate the virus, the treatment slightly reduced seed germination. None of the control treatments (without PEG) was successful in freeing seeds from the active virus.

Discussion

Since, LMV incidence in the field is largely dependent upon the amount of seed-borne virus [10], attempts in the present study, were made to erradicate LMV carried in the seeds. Results of treating LMV-infected lettuce seeds of the two local cultivars, Balady and Eskandrany with dry heat showed that exposure of infected seed to 80°C for 3-4 days was effective in eradicating the virus. However, the treatment slightly reduced seed germination. Nearly the same conclusion was reached by Howles [11]. A reduction in the level of LMV-infection by heating dry lettuce seeds to over 100°C has been reported but the treatment reduced germination [12].

When seeds were imbibed in PEG and heated at 40°C for 10 days, complete inactivation of the virus in both cvs. with no effect on seed germination, was achieved. These results, in general, agree with those obtained by Walkey and Dance [13] who reported that LMV was inactivated in PEG imbibed seeds incubated continuously at 40°C for 6-10 days. Our results showed that exposure to 40°C for 5 days did not greatly affect seed transmission of the LMV-Alex isolate and thus required a longer exposure period for inactivation. The successful inactivation of LMV in PEG-treated seeds is possibly due to the controlled imbibition allowing the processes of virus synthesis to start, thereby making the virus susceptible to the high temperature treatment [13].

In the light of the information obtained in this paper, one could safely recommend the use of PEG treatment coupled with heating at 40°C for 10 days to eradicate LMV from lettuce seeds in Egypt. This treatment which eliminates the primary source of infection is expected to reduce losses due to the disease.

Table 3. Effect of polyethylene glycol coupled with heat treatment on eradication of lettuce mosaic virus carried in Eskandrany lettuce seeds.

Treatment	Incubation period (days)	Temperature (°C)	Seed germination		Seed transmission		Incubation period means		Temperature means		Treatment means	
			%	Trans.[a]	%	Trans.[b]	a	b	a	b	a	b
PEG	5	22	99.8	88.4	7.7	15.8						
		38	99.1	84.5	6.4	15.0	84.7	14.8				
		40	96.6	81.1	5.6	13.6						
	10	22	99.6	87.8	5.5	13.5			83.1	13.2		
		38	98.2	82.3	4.0	11.5	84.0	8.6	78.7	11.7	79.7	9.9
		40	98.0	81.9	0.0	0.7			77.2	5.0		
	15	22	91.5	73.1	3.1	10.2						
		38	86.3	69.4	2.0	8.7	70.4	6.5				
		40	84.9	68.6	0.0	0.7						
Control (Without PEG)	5	22	100.0	90.0	7.3	15.8						
		38	98.0	81.9	5.7	14.6	84.6	14.9				
		40	98.0	81.9	5.1	14.2						
	10	22	99.8	88.4	5.7	13.7			87.7	14.3		
		38	98.7	84.7	4.2	11.8	85.0	11.8	82.2	12.7	83.4	12.8
		40	98.0	81.9	2.9	9.8			80.2	11.4		
	15	22	99.1	84.6	5.6	13.3						
		38	96.9	80.0	4.3	11.8	80.4	11.7				
		40	94.8	76.7	3.7	10.1						

$L.S.D._{0.05}$	Seed germination	Seed transmission
Treatment	1.6	0.7
Incubation period	2.3	0.9
Temperature	2.3	0.9

Trans.[a] and trans.[b] are the transformed values corresponding to seed germination and virus seed transmission percentages, respectively. To allow statistically analysis, data as percentages were first transformed as described by Snedecor [9, P. 327]. Data are average of 3 replicates.

Table 4. Effect of polyethylene glycol coupled with heat treatment on eradication of lettuce mosaic virus carried in Balady lettuce seeds.

Treatment	Incubation period (days)	Temperature (°C)	Seed germination		Seed transmission		Incubation period means		Temperature means		Treatment means	
			%	Trans.[a]	%	Trans.[b]	a	b	a	b	a	b
PEG	5	22	98.1	82.2	6.5	14.8						
		38	94.5	77.3	6.1	12.3	78.5	13.1				
		40	93.5	75.9	4.4	12.1						
	10	22	96.5	79.3	4.7	12.5			77.5	11.8		
		38	93.3	75.0	4.1	11.7	76.1	8.3	73.8	10.4	74.7	8.9
		40	92.4	74.1	0.0	0.7			72.9	4.5		
	15	22	89.3	71.0	2.1	8.2						
		38	87.3	69.1	1.6	7.2	69.6	5.4				
		40	86.1	68.6	0.0	0.7						
Control (Without PEG)	5	22	97.1	82.5	6.0	12.3						
		38	97.1	80.2	4.2	11.8	80.7	11.8				
		40	96.5	79.3	3.8	11.2						
	10	22	98.1	82.5	5.6	13.6			82.2	12.8		
		38	97.1	80.2	4.4	12.0	80.4	12.1	80.0	11.8	80.1	11.8
		40	96.1	78.6	3.4	10.8			78.2	10.9		
	15	22	97.9	81.7	4.6	12.4						
		38	96.7	79.5	4.0	11.7	79.3	11.6				
		40	94.8	76.8	3.4	10.8						

$L.S.D._{0.05}$	Seed germination	Seed transmission
Treatment	0.8	1.0
Incubation period	1.1	1.4
Temperature	1.1	1.4

Trans.[a] and trans.[b] are the transformed values corresponding to seed germination and virus seed transmission percentages, respectively. To allow statistically analysis, data as percentages were first transformed as described by Snedecor [9, P. 327]. Data are average of 3 replicates.

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العلاج الحراري لبذور الخس المصابة بفيروس موزايك الخس

جابر أحمد إبراهيم فجله، السيد السيد وجيه، يحيى محمد حامد الفحام
و حافظ أحمد الكريوني
قسم أمراض النبات، كلية الزراعة، جامعة الإسكندرية، مصر

ملخص البحث. أوضحت الدراسة التي تم فيها معاملة بذور الخس المصابة بفيروس موزايك الخس بالحرارة الجافة على درجة ٤٠، ٥٠، ٦٠، ٧٠، ٨٠، ٨٥°م لمدة ١، ٢، ٣، ٤ أيام أن التعريض لدرجة حرارة ٨٥°م لأي من الفترات المذكورة أدى إلى القضاء على الفيروس ولكن هذه المعاملة أدت إلى انخفاض إنبات البذرة بدرجة كبيرة. إلا أن معاملة البذور على درجة حرارة ٨٠°م لمدة ٣ أو ٤ أيام كانت فعالة في إبادة الفيروس بدون تأثير معنوي على إنبات البذور. كما بينت الدراسة التي تم فيها تشريب البذور المصابة بالفيروس بإبادة البولي إيثيلين جليكول وتسخينها على درجة حرارة ٢٢، ٣٨ أو ٤٠°م لمدة ٥، ١٠ أو ١٥ يوم أن الفيروس في بذور كلا الصنفين بلدي وإسكندراني تم القضاء عليه فقط عند معاملة البذور بالبولي إيثيلين جليكول وتسخينها على درجة حرارة ٤٠°م لمدة ١٠ أيام.