4

77

BARD

FINAL REPORT

PROJECT No. I-133-79

Resistance and Interference Phenomena in Plant-Virus Interactions

G. Loebenstein, A. Gera, A. Stein, S. Spiegel,
M. Chessin

27

LOE

1983

1895 0

Standard BARD Cover Page for Scientific Reports

Date July 1983

BARD
P.O. Box 6
Bet Dagan, ISRAEL

BARD Project No. I-133-79

Title of Research Project:

Resistance and Interference Phenomena in Plant-Virus Interactions

Names of Investigators:

Principal Investigator: Prof. G. Loebenstein, A. Gera, Dr. Adina Stein, Dr. Sara Spiegel

Cooperating Investigator(s): Prof. M. Chessin

Name and Address of Affiliated Institutions:

Principal Institution: Agricultural Research Organization, Volcani Center,
Bet-Dagan, Israel

Cooperating Institution(s): Dept. of Botany, University of Montana, Missoula,
Montana, 59812

Project's starting date: May 1980

Type of Report: 1st Annual 2nd Annual Final X

Signatures:

Principal Investigator

J. Loebenstein

Institution's Authorizing Official

Yehud Eshdat

Dr. YUVAL ESHDAT

Head, Div. for Research Projects and Budgets
Agricultural Research Organization

Virus diseases of plants of plants - Diseases
and pests resistance

$$\frac{630.72}{\text{BAR}} / 632.8 : 581.29$$

2nd copy

Table of Contents

a. Cover page	1
b. Table of Contents	2
c. Abstract	3
d. Body of Report	4
A. <u>The Use of Plant Protoplasts in Studies of Resistance</u>	4
(1) Effect of 2,4-D on TMV	4
(2) Inhibitor of virus replication (IVR)	8
(3) Enhanced TMV production in the presence of antibiotics	9
(4) Suppression of TMV replication at increasing mannitol concentrations	13
(5) Evaluation of IVR on animal viruses	14
B. <u>Studies of Resistance in Intact Plants</u>	15
(1) Changes in ribosomal fractions associated with induced resistance	15
(2) Changes in ribosomal fractions in CMV and PVX resistant tissues	16
(3) Effect of EMA in several virus-host combinations	18
(4) Effect of EMA on protein profiles	20
(5) Serological specificity of ribosomal preparations	21
C. <u>Photobiology</u>	24
(1) UV on systemic acquired resistance	24
(2) UV on EMA induced resistance	25
(3) UV on delocalization	29
(4) Effect of time intervals	31
(5) UV on localized acquired resistance	33
D. <u>Inhibitor from Datura Stramonium</u>	34
E. <u>Effect of Aspirin on TMV Replication</u>	37
e. Evaluation of Research Achievements with Respect to Original Proposal	39
f. Cooperation	39
g. Thesis	40
h. Benefit to Agriculture	40
i. Reprints	40

Abstract

The objectives of this project were to get a better understanding of resistance mechanisms in plants to viruses, and to see if these are associated with natural antiviral substances.

It was shown that a true inhibitor of virus replication (IVR) is associated with the local lesion response, IVR was obtained from tobacco mosaic virus- infected protoplasts. IVR was partially purified and yielded two biologically active principles of about 26,000 and 57,000 daltons. IVR was found to be sensitive to proteolytic enzymes, but not to RNase and seems to be of a proteinaceous nature. IVR inhibited virus replication in protoplasts, leaf tissue disks and when applied by spraying to intact plants. IVR inhibited several plant viruses and seems not to be virus or host specific. It was also found that TMV multiplication in protoplasts is suppressed at increasing mannitol concentrations.

Different forms of inducing resistance in the intact plants were compared. When resistance was activated most intensely a change in ribosomal fractions became evident. No consistent effect on leaf protein profiles was obtained using a polyanion for inducing resistance.

Ultraviolet (UV) irradiation markedly increased local lesion size when applied up to 24 hr after inoculation. However, UV had no effect on systemic acquired resistance (SAR) indicating that localization and SAR differ in their mechanism.

An inhibitor of infection from Datura and the effect of aspirin on acquired resistance were also studied.

d. BODY OF REPORT

The research was centered around 5 major aspects.

A. The Use of Plant Protoplasts in Studies of Resistance

(1) Effect of 2,4-D on TMV growth curves in protoplasts

TMV growth curves in protoplasts from resistant (Samsun NN and Xanthi-nc) and in systemic (Samsun) varieties, as affected by 2,4-D in the medium were determined.

Protoplasts were obtained from the first upper two almost fully expanded leaves of N. tabacum L. cv. Xanthi-nc and NN plants, 5 to 6 weeks after planting. Plants were kept in the greenhouse. Three feedings with a complete nutrient solution were given at weekly intervals, starting 1 week after planting, and leaves 9-12 from the top were selected 13-21 days after the last feeding. The mixed enzyme procedure, with 0.05-0.1% macerozyme R-10 and 0.5-0.8% cellulase R-10 (according to season) in incubation medium containing 13% (w/v) mannitol, 0.2 mM KH_2PO_4 , 1 mM KNO_3 , 0.1 mM MgSO_4 , 10 mM CaCl_2 , 1 mM KI, and 0.01 μM CuSO_4 (VIM) (Loebenstein et al., 1977) was used. For washing of protoplasts from debris, the procedure outlined previously (Loebenstein et al., 1977) was followed. The infection medium consisted of 0.02 M potassium citrate buffer pH 5.2, containing 13% mannitol, poly-L-ornithine (Sigma, approx. MW 120,000) (PLO), and TMV purified by density gradient centrifugation. Various concentrations of PLO and TMV were tested as outlined under Results. The infection medium was incubated at 25° for 10 min while being rocked gently (32 oscillations/min). Ten milliliters of the medium was then added to 10 ml of mannitol (13%) containing 5×10^6 protoplasts. The mixture was incubated at 25° for

10 min while being rocked gently. Inoculated protoplasts were then centrifuged from the infection buffer at 12 g for 6 min, washed twice with 13% mannitol containing 0.1 mM CaCl_2 and once with VIM containing carbenicillin at 200 $\mu\text{g/ml}$ and mycostatin at 25 units/ml. The protoplasts were then divided into two lots. One was suspended in VIM containing the antibiotics and 2,4-D (1 $\mu\text{g/ml}$) (Sigma), and the other without 2,4-D. Protoplasts were divided into 100-ml Erlenmeyer flasks at a concentration of 1×10^5 protoplasts/ml, and incubated at 25° under continuous illumination of about 2000 lx. Protoplasts sampled after 96 hr of incubation were used to determine the number of infected protoplasts by the fluorescent antibody technique (Loebenstein et al., 1977).

To obtain protoplasts of N. tabacum cv. Samsun leaves the procedure was similar, except that enzyme concentrations were 0.05-0.12% macerozyme R-10 and 0.2-0.5% cellulase R-10. Mannitol at a concentration of 13.5% was used in all media.

As difficulties in sedimenting Samsun protoplasts from VIM by centrifugation were encountered, all suspensions were harvested for infectivity assay by suction filtration onto two 5-cm Whatman GF/C glass-fiber paper disks, following counting in a hemocytometer. The disk with the protoplasts was homogenized for 2.5 min in 4 ml of 0.05 M phosphate buffer, pH 7.5, in the cold, with the aid of a microattachment to the Omni-Mixer. The homogenate was inoculated onto 12 half-leaves of N. glutinosa plants and compared to a standard solution of purified TMV on the opposite half-leaf. Lesion counts were adjusted to 10^6 live protoplasts and calibrated using the following equation:

calibrated number of lesions per half-leaf for homogenate

mean number of lesions per half-leaf produced by homogenate

=

mean number of lesions produced by standard TMV on opposite half-leaf

X mean number of lesions per half-leaf produced by standard TMV from all experiments.

RESULTS

Tobacco mosaic virus (TMV) multiplication was markedly enhanced in protoplasts from Samsun NN and Xanthi-nc, two local-lesion-responding cultivars, by 2,4-dichlorophenoxyacetic acid (2,4-D) added to the incubation medium after inoculation of the protoplasts. In Samsun, a cultivar which reacts systemically to TMV infection, 2,4-D reduced virus multiplication. (Table 1).

These findings may explain the discrepancy observed between the low virus multiplication in intact tobaccos with the N gene and the high titers reached in protoplasts from these plants (in the presence of 2,4-D), which were similar to those found in protoplasts from a systemic cultivar.

Loebenstein, G. et al. (1977). Virology 81:117-125.

Table 1. Effect of 2,4-D in incubation medium (with sucrose) on extractable TMV from Samsun NN and Samsun protoplasts incubated at 22°C and harvested by centrifugation^a

Hours of incubation after inoculation ^b	Infectivity ^c		Ratios, 2,4-D treated to untreated
	with 2,4-Dd	without 2,4-D	
	Samsun NN		
48	10.2 ± 1.53	4.0 ± 1.14	2.6
72	31.8 ± 4.68	14.6 ± 3.33	2.2
96	55 ± 3.83	25.2 ± 1.42	2.2
	Samsun		
72	34 ± 2.15	87.1 ± 6.11	0.39
96	65.4 ± 4.93	108.3 ± 3.00	0.60

^a Average from 3-4 experiments

^b Infection medium contained 1 µg/ml poly-L-ornithine. VIM contained 13.5% mannitol and 1.5% sucrose

^c Average number of lesions and standard error per 10⁶ protoplasts on one half-leaf of N. glutinosa calibrated to standard TMV solution (2.5 µg/ml; about 60 lesions per half-leaf).

^d 2,4-D, 1 µg/ml.

(2) Influence of UV on EMA-induced resistance to infection of
necrotic tobacco

The injection of the polyanion EMA (see section B (1) of report) into leaves of Samsun NN tobacco induces resistance to TMV, decreasing both lesion number and lesion size. It was therefore of interest to see the effect of UV on the development of this type of resistance.

EMA 0.5 mg/ml was injected into the intercellular spaces of the test leaves on intact plants. These were kept for 24-96 hrs in the green house, where the leaves were exposed to UV at a dose rate of 232 nm for varying lengths of time. Plants were kept then in the dark from 16-30 hrs to minimize photoreactivation after which they were inoculated with purified TMV. Local lesion numbers and areas were determined 5-6 days after inoculation.

Results are summarized below

Experiment 1. Xanthi nc tobacco, 32 hours between EMA and UV treatments, 16 hours dark after UV.

total leaves - 112

A-4 replicates

B-3 replicates

Results

UV dose (mins)	ave. % reduction lesions compared to controls EMA		UV		EMA+UV		sign test	
	no. /	ave. area (mm ²)	no.	area	no.	area	no.	area *
<u>A</u>								
1.5	56	38	31	4	69	22	-	-
2.5	64	9	64	-9	71	7		+
3.0	68	56	56	5	79	57		-
<u>B</u>								
1.0	17	54	0	4	23	41	+	-
1.5	16	44	41	3	57	45	0	-
2.0	66	28	0.3	18	63	54	-	+
3.0	9	73	7.7	2	88	72	+	-

* + indicates a greater reduction than expected from additive effects of EMA and UV effect on capacity.

- indicates a lesser reduction than expected, therefore suggesting a partial reversal by UV of the EMA-induced resistance. No sign test applied where additive reduction of EMA and UV separately were greater than 100%.

Experiment 2. Xanthi nc tobacco.

- a) 48 hours between EMA and UV, 16 hours dark after UV
7 replicates
112 leaves
- b) 88 hours between EMA and UV, 16 hours dark after UV
2 replicates
32 leaves

Results

		<u>% reduction</u>						sign test	
		EMA		UV		EMA+UV			
	<u>dose</u>	<u>no.</u>	<u>size</u>	<u>no.</u>	<u>size</u>	<u>no.</u>	<u>size</u>	<u>no.</u>	<u>size</u>
a)	1	50	7	40	3	70	38	-	+
	1.5	62	12	37	2	75	21	-	+
	2.5	37	5	0	1	50	3	+	-
b)	2.0	68	47	25	-0.5	78	49	-	+

- Experiment 3. Burley 21 tobacco, 24 hours between EMA and UV, 30 hours dark after UV.
2 replicates
32 leaves

Results

		<u>% reduction</u>						sign test	
		EMA		UV		EMA+UV			
	<u>dose</u>	<u>no.</u>	<u>size</u>	<u>no.</u>	<u>size</u>	<u>no.</u>	<u>size</u>	<u>no.</u>	<u>size</u>
	2.5	69	38	41	25	83	37.5		+

Experiment 4. Samsun NN tobacco, 24 hours between EMA and UV treatments, 24 hours dark after UV.

5 replicates

80 leaves

Results

a. upper leaf surface irradiated and inoculated

dose (sec.)	EMA		UV		EMA+UV		sign test	
	no.	size	no.	size	no.	size	no.	size
5	86	37	91	-32	95	53		+
10	62	5	27	11	85	41	-	+
20	95	88	5	-1	96	89	-	+
40	93	74	33	42	94	85		
60	92	66	30	21	96	67		+
90	85	78	53	38	95	67		
150	86	80	60	62	92	82		

b. upper leaf surface irradiated, lower surface inoculated

5	85	43	50	-65	79	-40		
10	68	10	46	14.5	91	18		-

Experiment 5. Samsun NN tobacco, 48 hours between EMA and UV, 24 hours dark after UV.

3 replicates

48 leaves

Results

dose (mins)	EMA		% reduction		EMA+UV		sign test	
	no.	size	no.	size	no.	size	no.	size
2.5	89	73	26	50	91	60		
3.5	88	95	15	50	97	68		

Experiment 6. Samsun NN tobacco, 96 hours between EMA and UV, 24 hours dark after UV treatment.

2 replicates

32 leaves

Results

dose (mins)	EMA		% reduction		EMA+UV		sign test	
	no.	size	no.	size	no.	size	no.	size
1.0	94	60	56	18	97	83		+
1.5	95	69	53	46	95	75		
2.0	92	51	57	42	94	81		-
3.0	97	67	42	40	95	57		

Conclusions

1. On the basis of the sign test, there is a tendency for UV to partially reverse EMA-induced resistance with respect to lesion numbers, but to enhance resistance as far as lesion size is concerned.

(3) Effect of UV irradiation on delocalization

The effect of UV irradiation on delocalization was tested in several host inoculated with TMV. Experiments were performed using $\frac{1}{2}$ -leaf irradiations. Plants irradiated 22 hours after inoculation, and darkened for 24 hours after UV.

Experiment 1. Xanthi nc.

2 replicates

24 leaves

November 19-November 28, 1981

dose (min.)	UV		Effect on Lesions control	
	no.	size	no.	size
2.5	13.5	2.0	22	2.0
5.0	7.6	1.96	12.0	2.0
10.0	5	1.45	17.3	3.0

Experiment 2. Samsun NN

2 replicates

16 leaves

September 28-October 3, 1981

dose (min)	UV		control	
	no.	size	no.	size
1	146	2.3	164	2.3
2	117	2.4	176	2.5
3	40	2.4	86	2.4
4	73	2.4	172	2.5
5	62	1.9	140	2.1
6	6	1.5	32	2.1
7	12	1.7	75	2.5

Conclusions

1. At higher doses, UV decreased both size and number of lesions .
Some carryover to unirradiated half-leaves was noted.
2. There was no evidence of UV-induced delocalization in either tobacco variety. Consequently, we tested our system with Pinto bean which is known to respond with delocalization.

Experiment 3. Pinto bean

1 replicate

40 leaves

opposite primary leaf technique

<u>dose(sec.)</u>	UV		control	
	<u>no.</u>	<u>size</u>	<u>no.</u>	<u>size</u>
10	47	0.80	38	0.80
20	18	0.82	19	0.80
30	268	0.70	121	0.45
60	600	0.71	220	0.40
90	312	0.70	111	0.45

Conclusions

1. At overlapping doses to those used with tobacco, we obtained excellent delocalization with bean.
2. This unique failure of tobacco varieties to respond to UV deserves further investigation.

(4) Effect of interval between TMV infection and UV treatment on delocalization and systemic acquired resistance (SAR) in Pinto beans

Time intervals between inoculation and irradiation were tested. Challenge inoculations were done on whole or half leaves (depending on whether whole or half leaves were used for primary inoculations) one week after the primary inoculations. The effect of UV on lesion numbers and areas were determined. SAR was assessed by comparing challenged whole or half leaves opposite their virus-infected counterparts with those opposite buffer-rubbed whole or half leaves.

Results are summarized in the following table

Effect of interval between virus infection and UV treatment on delocalization and SAR

Interval V→UV (days)	Delocalization				SAR (^V /B) ***			
	no		area		no		area	
	* W	** H	W	H	W	H	W	H
0 (no UV)	3.3	2.2	0.10	0.05	1.1	0.8	0.7	2.0
1	4.2	4.6	0.20	0.11	0.3	0.6	0.7	0.8
2	4.9	5.3	0.16	0.08	0.5	0.7	0.9	0.9
3	4.7	5.6	0.13	0.08	0.5	0.9	0.9	0.9
4	4.9	3.5	0.15	0.08	1.5	0.9	0.9	0.9

* whole leaf challenge

** half-leaf challenge

*** virus to buffer

Comments

1. UV again produced a marked delocalization both with respect to size and number of lesions, although there was a greater effect with the greater time intervals than had been previously reported.
2. UV had no effect on SAR at any time interval, although the magnitude of SAR was either disappointingly low or variable in this experimental series.

(5) Effect of UV irradiation on localized acquired resistance (LAR)

Since UV causes ~~de~~localization and increases in virus titer in some species, we were interested in determining whether it had any effect on LAR.

Experimental Samsun NN, 3 replicates

30 leaves, Aug. 8-Nov. 13, 1981.

Primary inoculation with virus at 1/120 dilution of clarified infectious sap, irradiated with UV 24 hours later, then 24 hours dark treatment, followed by challenge inoculation 5-6 days later with purified virus at 0.3 mg/ml. Lesions recorded 5 days later. Numbers and sized of challenge lesions in a circular zone of 113.1 mm² area around primary lesions were compared to comparable control areas in portions of leaves which did not receive primary inoculation

Results

% reduction in lesions in resistant zones as compared to controls

<u>dose (min.)</u>	<u>No UV</u>		<u>UV</u>	
	<u>no.</u>	<u>size</u>	<u>no.</u>	<u>size</u>
1	80	85	91	89
1.5	96	93	95	95
2.0	64	96	82	95
3.0	71	94	35	99

Conclusions

1. LAR strongly demonstrated in our system.
2. UV did not reverse LAR. Only in one instance (3 min UV) did UV reverse LAR but with respect to lesion numbers only.
3. To complete this study, we intend to study the possible effect of UV on virus titer in resistant zones.

In summary

Ultraviolet light (UV) markedly increase size of local lesions produced on leaves of Pinto beans, when they were irradiated 24 hr after inoculation with TMV. However, UV had no effect on systemic acquired resistance (SAR). These results indicate that virus localization and acquired resistance are not based on identical mechanisms (Chessin, 1978).

D. Inhibitor from Datura stramonium

Materials and Methods

a. Ammonium Sulfate fractionation:

Successive precipitation of the inhibitor were carried out using $(\text{NH}_4)_2 \text{SO}_4$ (AS) at 20, 40, 60, 80, and 100% saturation. Precipitates were centrifuged, dialyzed overnight in a cold room against standard bi-phosphate buffer pH-7.0. The buffer was changed at 4 hr and 6 hrs intervals. Dialyzates were tested for inhibitory activity. The inhibitory activity was measured by mixing the AS fraction 1:1 with TMV and inoculated by finger rubbing onto Pinto bean leaves. Lesion size and number were measured. Controls consisted of crude Datura sap 1:1 with TMV and TMV 1:1 with standard buffer.

b. Sephadex G-75 fractionation

The AS fractions containing significant inhibitory activity were separately placed on the column. Alternate collecting tubes were analyzed for A_{280} and inhibitory activity as above.

c. Hydrated Calcium Phosphate (HCP)

HCP was mixed 1:1 with the semi purified Datura Sap (SPDS) and centrifuged at 3000 rpm for 15 min. The supernatant was again mixed

with TMV and inoculated by finger rubbing onto Pinto bean.

d. Biological activity of SPDS

1. Detached leaves.

Pinto bean half leaves were inoculated with SPDS + TMV (1:1). Leaves were detached and placed in petri dishes with three layers of wet filter paper. The petri dishes were placed in a growth chamber with optimum light and temperature for 5 days. Controls consisted of TMV + standard buffer (1:1). Attached Pinto bean leaves were also tested. Lesions number and size were measured.

2. Time of application

Half leaves of Pinto bean leaves were inoculated with either TMV or SPDS. One hour later they were rinsed with distilled water, blotted dry with paper towels and subsequently inoculated with TMV, SPDS or buffer. Lesions number and size were measured.

Results and Discussion

Significant inhibitory activity was obtained from AS fractions 21-80%. No effect on lesion size was apparent in any of the fractions.

The Sephadex (SX) fractions with the highest inhibitory activity was the 50% AS, giving 99% inhibition when mixed with TMV (Table 1).

Table 1: Inhibitory activity of the Ammonium sulfate fractions
placed on Sephadex G-75 column.

<u>AS fraction</u>	<u>SX fraction</u> (tube #'s)	<u>% Inhib (based on lesion#)</u>
40%	12-23	52
(21-40%)	24-36	24.8
	37-44	36.7
	45-61	20.8
	62-74	-10.7
60%	13-33	59.8
(41-60%)	34-71	-71.1
80%	15-21	99
(61-80%)	22-31	65.8
	32-41	58.5
	42-65	-.05

Based on 8 half leaves/SX fraction

It seems that the endogenous inhibitor probably precipitates between 61-80% saturation of AS. The appearance of the inhibitory activity in the void volume of the column tentatively suggests a high molecular weight.

Using hydrated calcium phosphate (HCP) for further purification of the inhibitor does not precipitate much inhibitor activity.

The inhibitory activity of SPSP was stable when frozen for more than 10 months.

To see whether the decrease in lesion numbers in the presence of SPDS may be due to a lack of visibility, i.e. microlesions that were too small to be counted, the biological activity of SPDS was tested on detached and attached leaves knowing that detached inoculated leaves increases virus lesion size, any microlesions would expand to countable size. A four-fold increase in lesion size (based on TMV controls) was observed and yet percentage of inhibition remains the same (Table 2).

Table 2. Effect of SPDS on lesion size in detached and attached Pinto bean leaves.

(based on lesion #)			
<u>Condition</u>	<u>Treatment</u>	<u>% Inhibition</u>	<u>Avg. Lesion Size (N)</u>
Detached	TMV + SPDS	99%	4 mm (1)
	TMV + Buffer		68 mm (70)
Attached	TMV + SPDS	100%	0
	TMV + Buffer		

Thus the decrease in lesions number is not due to the masking of lesions by their small uncountable size. Also SPDS appears to have an effect on virus infection and not virus replication. The application of SPDS immediatly or 1 hr after the infection with TMV does not inhibit virus replication, the virus establishment was inhibited.

E. Effect of Aspirin on TMV Replication

Aspirin solutions were applied to tobacco on Pinto bean plants either by watering or spraying at a concentration of 0.05% or by leaf injection at 0.02%. All were adjusted to pH 6.5 and 1% Tween 20 added to solutions used as sprays. Control plants sprayed with water.

Aspirin solution (0.05%) was injected into leaf intercellular spaces of Xanthi nc. tobacco at different intervals before and after inoculation with TMV. Control leaves were injected with buffer. One week after inoculation, lesions were counted and their areas were measured.

Results and Discussion

Aspirin clearly affects virus establishment in tobacco when applied less than 72 hr before or no longer than 24 hr after inoculation, based upon its effects on lesion number (Table E-1).

Table E-1. Effect of aspirin on lesions number and area, when applied
to Xanthi nc leaves different times before and after inoculation

<u>Treatment</u>	<u>Interval (hr)</u>	<u>% change in</u>	
		<u>number</u>	<u>area</u>
Aspirin before virus	24	-20	-55
	48	-14	-46
	72	+24	-51
	96	+45	-27
Virus before aspirin	0	- 5	-23
	4	- 5	-43
	6	- 2	-38
	8	-17	-57
	12	- 3	-27
	24	- 1	-13

Aspirin consistently reduced lesion area when applied before or after infection. Although if applied 24 hrs after, the effect seems to be wearing off.

No reliable results were obtained with Pinto beans treated with aspirin.

e. Evaluation of Research Achievements with Respect to Original Proposal

The research closely followed the original research proposal. Three major lines of research were originally outlined: A. The use of protoplasts in studies of resistance; B. Studies of resistance in the intact plant; and C. Photobiology of acquired resistance. Within these three areas the main emphasis - originally and due to the very positive results - was given to A, and mainly to A 1, 2, 3 and 4 relating to the inhibitor of virus replication (IVR) released from resistant protoplasts. The main achievement - the detection and characterization of IVR was in the center of the original proposal; while within B several lines of research, as B-3 were not fruitful.

f. Cooperation

During the three years research results, including manuscripts, were exchanged and evaluated being the basis for the adaptation of the program. Opportunity for an intensive exchange and evaluation of research data, and planning future research was given when G. Loebenstein visited Dr. Chessin in Montana. As a result a new joint BARD proposal was submitted and additional lines of research based

on the present cooperation, were considered by the two groups. Thus, Dr. Chessin will become involved in virus-protoplast studies and Loebenstein's group will use UV techniques in several resistance studies.

g. Thesis

Within this project A. Gera completed his Ph.D. thesis entitled: "Partial purification, characterization and biological properties of an antiviral substance from tobacco mosaic virus-infected protoplasts of a local-lesion responding tobacco" submitted and approved by the Tel-Aviv University.

h. Benefit to Agriculture

Benefits to agriculture are in the long range. Understanding natural resistance mechanisms is presently limited and may provide in the future ways of controlled induction of resistance. The finding that localization (resistance) is associated with a substance acting as an inhibitor of virus replication (IVR) also when applied by spraying to intact plants may open new possibilities for the control of plant viruses. This may lead in the future to the development of an antiviral compound, perhaps by genetic engineering techniques. These studies will probably lead in the future to the isolation of the gene responsible for resistance and its introduction into susceptible plant.

i. Reprints

Reprints and manuscripts are included as part of section d.