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Title of Project

APPLICATION OF RAPID TECHNIQUES TO VIRUS CERTIFICATION IN FRUIT TREES

Principle investigator : Dr. Sara Spiegel
Grantee Institution : The Volcani Center, Israel

Collaborator : Ing. Mustapha Zemzami
Institution : Direction des Domaines Agricoles, Morocco

Project number: C16-059

USAID Grant Project Officer: Mr. William H. Crane

Project Duration: April 97 - September 2000

Executive Summary

The main goal of this project is to facilitate production of virus-tested propagation material and shorten virus detection procedures for the rosaceous fruit-tree industry in Morocco and Israel. The project is focusing on cultivars and selections of almond and apple of local interest and advantage. The targeted viruses are: prunus necrotic ringspot (PNRSV); prune dwarf (PDV) and apple chlorotic leaf spot (CLSV).

Since the previous annual report, the following research acitivites were performed:

- The virus-infected, in vitro grown, almonds collection, established in Morocco in the first year was expanded and includes now selections from the salt-tolerant seedling population in the Atlas mountains region. Local selections of apple cultivars, were established in vitro, for the first time, in Morocco. Micro-propagation of almond and apple cultivars, selections, and woody indicators is currently operating in Morocco.
- Virus elimination protocols including thermotherapy, chemotherapy (using virazole) and combined treatments were performed in Israel with PNRSV-infected almond and plum cultivars and CLSV-infected apple. Local, Israeli selections were included. Regenerates are being tested for virus presence by ELISA and by PCR for 10-12 months.

In Morocco, shoot tip grafting (STG), has been studied as an alternative virus elimination method for stone fruit hosts. A comparison of several rootstocks used for grafting the excised shoot-tip showed clearly that the selection and use of the right rootstock affects the success rate dramatically. For almond, GF305 and the local Pecher de Missour were the best, while GF677 was a poor rootstock for STG. An improvement of the STG technique was obtained by using rootstocks severed from greenhouse grown plants rather than in vitro grown one. Greenhouse indexing was studied in both labs. In Morocco, Pecher de Missour plants were evaluated as a new woody indicator for the targeted viruses. Preliminary results show that plants react with symptoms to graft inoculation with PNRSV and PDV but not to CLSV. In Israel, micro-propagated GF305 and Pyronia plants have been used for greenhouse indexing studies. Problems encountered previously in the hardening stage were overcome.

Collaboration between the groups in Morocco and Israel has been very good and intensive, information is transferred constantly and communication is frequent. A MERC proposal was recently submitted by the partners of this project (UCP and VC groups) and two additional labs.

Section I

A. Research Objectives:

The general goal of this project is to facilitate production of virus-tested certified propagation material and shorten virus detection procedures for the rosaceous fruit-tree industry in Morocco and Israel. The specific immediate objectives are:

a. Develpment of in vitro therapy protocols for selected virus-infected almond and apple cultivars.
b. Development of sensitive detection assays for fruit-tree viruses, based on:
   1. Target sequence amplification molecular techniques.
   2. Short-term, year-round, biological indexing.

The targeted viruses are - prunus necrotic ringspot (PNRSV), prune dwarf (PDV) and apple chlorotic leafspot (CLSV).

B. Research Accomplishments

1. Collection of plant material and establishing a collection of virus-infected fruit tree cultivars and rootstocks (Morocco, Israel)

In Morocco, in addition to last year's list, five selections from the salt tolerant almond seedling population in Toundout (South-East Atlas Mountains) have been established under greenhouse (#2, #6, #10, #11 and #19 in the Almonds picture we submitted in MERC proposal M20-072). In vitro cultures established previously in the frame of this project were propagated and virus status determined as detailed below in Table 1.

In Israel, micropropagation of GF305 aimed to produce young indicators for year-round indexing was undertaken. Proliferation was achieved using the Almehdi & Parfitt medium descibed previously. However, difficulties in rooting and hardening were encountered during the first year. During the second year, a protocol for rooting in vitro grown GF305 shoots (BDR medium, following Desvignes, CTIFL, Lanxsade Center, France) and a hardening procedure allowed transferring plants to the greenhouse and growing vigorous potted plants. GF305 seedlings have been used for (1) evaluation of greenhouse indexing (described below) and (2) establishment of a collection of PNRSV and PDV isolates

GF305 plants have been used to establish a collection of virus isolates in addition to those kept in original hosts (when possible). Chip/bud samples of virus infected (PNRSV, PDV, CLSV) trees have been grafted on young GF305 seedlings. Infectivity was validated by symptoms and ELISA assays. The advantage of the GF305 collection was demonstrated with the rose isolate of PNRSV. Whereas ELISA assays were positive using rose leaf or bark tissue, RT-PCR assays were negative. Transmitting this virus isolate to GF305 allowed a positive RT-PCR assay.

Table 1. Summary of in vitro cultures of rosaceous fruit tree species and indicators in the UCP lab (Rabat)

Almond	Virus status	No.	origin
Ferraduel	PNRSV, PDV, [PNRSV + CLSV]	235	Local introduction
Fournat	PNRSV, PDV, [PDV + CLSV]	173	Local introduction
Ferranies	[PNRSV + PDV]	44	Local introduction
Marcona	PNRSV, PDV, CLSV, [PNRSV + PDV]	179	
Tuono	PNRSV, PDV, [PNRSV + PDV]	197	
S2 du 2/7-II	?	34	(local introduction)
S2 du 2/7	?	12	(local introduction)
S4 du 2/7	?	40	(local introduction)
S1 du 2/7	?	31	(local introduction)
Chazanov	PNRSV	48	Obtained in vitro from VC
Samish	PNRSV	94	Obtained in vitro from VC
GF-677	healthy	131	Local introduction
Apple			
Golden Smouth	?	8	Local introduction
MM-106	?	55	Local introduction
Malus 70	CLSV	62	Obtained in vitro from VC
Ana 2	CLSV	19	Obtained in vitro from VC
Apple 24	CLSV	24	Obtained in vitro from VC
Ana 2	healthy	24	Obtained in vitro from VC
Malus 70	healthy	45	Obtained in vitro from VC
Plum			
Ogden	PNRSV	42	Obtained in vitro from VC
Indicators			
GF-305	healthy	19	Local introduction
Shirofugen	healthy	78	Obtained in vitro from VC
Pyronia	healthy	42	Obtained in vitro from VC
Virginia Crab	healthy	42	Obtained in vitro from VC
Charden	healthy	44	Obtained in vitro from VC

2. Adaptation of diagnostic bioassays, ELISA and RT-PCR for the targeted viruses (Israel, Morocco)

Reliable and rapid diagnostic and detection methods are an important component of virus elimination and, therefore, an effort was made to establish reliable and rapid protocols in both countries.

2.1 Bioassays

Greenhouse indexing, suitable for year-round testing is being studied with 2 indicators – GF305 peach seedlings and young Pyronia plants. GF305 is a general indicator exhibiting leaf symptoms with numerous viruses affecting rosaceous species and Pyronia develops leaf symptoms following infection with pome-fruit viruses.

GF305 peach seedlings - Graft inoculated plants, with bark samples from known infected PNRSV- and PDV trees and unknowns, usually showed typical symptoms on leaves within 4-6 weeks following grafting. Young Shirofugen plants were used to confirm infectivity with PNRSV and/or PDV, although not differentiating between the two viruses. Virus transmission to GF305 was also validated by ELISA assays. The reaction to CLSV is currently being studied.

Pyronia - The indicator Pyronia (for apple viruses), recommended for a range of pome fruit viruses by Ing. Desvignes (CTIFL, France) has been micro-propagated, rooted and transferred to the greenhouse for evaluation as a year-round indicator exhibiting leaf symptoms with a wide range of apple viruses. Graft–inoculation, with three known CLSV-infected apple cultivars and one rootstock, resulted in obvious leaf symptoms including mottle, tip necrosis and stunting of newly formed leaves. Greenhouse grafting will be continued according to the work-plan.

2.2 Evaluation of Pecher de Missour as an indicator for PNRSV, PDV and CLSV (Morocco)

Pecher de Missour (PM) is a local selection used as a drought tolerant rootstock in Morocco. The plant is vigorous and well adapted to local conditions. In addition to serving as a rootstock, a preliminary study was conducted to evaluate PM as an indicator plant for the above three mentioned viruses.

PM seedlings (germinated from seeds) were raised in pots in the greenhouse until the stem is 0.6 to 0.8 mm in diameter. The plants were graft-inoculated with chip/buds from healthy, PNRSV, PDV and CLSV woody plants in replicates of 10 for each virus and healthy control. Grafts were made at about 20 cm from the ground and cut at about 1 cm above inoculation point. Plants were watered and fertilized as needed and maintained for observation in the greenhouse.

While healthy controls and CLSV inoculated plants started to grow normally after cut-back, initiation of growth of PNRSV and PDV inoculated plants was delayed for 7 to 15 days and remained slower all the time. Two months post inoculation, all PNRSV plants and 8/10 PDV – plants exhibited characteristic symptoms. No difference in growth was noticed between healthy controls and CLSV inoculated plants and no symptoms of the latter were observed on any of the inoculated plants. The potential of PM as an efficient woody indicator for fruit tree viruses will be further studied.

2.3 ELISA

Assays, based on reagents purchased from Bioreba (Israel, Morocco) and Biochemica (Morocco) were used for detecting PNRSV and PDV. CLSV was not detected by ELISA with reagents from either source in Morocco. Following exchange of plant samples and reagents during visits, the system used in Israel (based on MABs from D. Thompson, Canada as second antibody) was found suitable also for Moroccan isolates.

2.4 RT-PCR

RNA-RT-PCR has been evaluated for detection of the three viruses. The assay for PNRSV was as reported by Spiegel et al., (1996). Protocols for PDV and CLSV were as published by Parakh et al., and (1995) Nemchinov et al., (1995) respectively. In Israel, detection of CLSV by RT-PCR was problematic and only recently, following an elevation of the annealing temperature resulted in a CLSV-specific amplified band. However, in our hands, on a routine basis this assay was not reliable.

3. Establishment of in vitro shoot cultures with double infections (Israel, Morocco)

Several almond cultivars with double infections were identified in Morocco (detailed in Table 1). In Israel, an attempt was made to obtain almond shoots infected with 2 viruses. For this purpose, almond (cv. Samish) shoots infected with PNRSV were micro-grafted with shoot tips (about 1 cm long) excised from CLSV- infected apple (Malus rootstock) shoots. Two orientations were used (1) almond served as 'rootstock' and apple as 'scion' and (2) apple served as 'rootstock' and almond as 'scion'. 25 shoots were used for each orientation. Passage of virus was monitored by ELISA assays for each of the two viruses in both 'rootstock' and 'scion'.

Results show that PNRSV was not transmitted from almond to apple. CLSV was transmitted from apple to almond only when apple served as 'rootstock' but not in the other orientation. The rate of transmission was very low (2/25 shoots). However, proliferation of these shoots allowed obtaining a shoot population with double infections for further studies. In the future, it is planned to study distribution of the two taxonomically viruses in shoots, therapy efficiency and multiplex PCR detection.

4. Thermotherapy, chemotherapy and combined treatments for in vitro virus elimination (Israel, Morocco)

Experiments aimed to develop effective therapy protocols were studied with virus-infected almond, plum and apple. Following treatments, shoot-tips (about 5 mm in length) were excised from the main shoot and newly formed axillary buds and planted on fresh medium for regeneration. Testing for viruses was done primarily by ELISA starting one to two months after the end of the experiment and repeated at intervals of two months for up to one year. PCR is used to confirm freedom from virus. Shoots are rooted, hardened and transferred to the greenhouse. It is intended to determine what is the minimum time required for reliable determination of virus status in regenerated plants. The plant materials, viruses, types of treatments and degree of success obtained in Israel are detailed below (Table 2).

Table 2. Plant materials, viruses and treatments in virus elimination experiments

Plant material	Virus	Treatment	Degree of success***
Almond Cv. 'Samish', 'Chazanov'	PNRSV	Heat treatment* 21 days 28 days	+ ++
Plum Cv. 'Ogden'	PNRSV	Heat treatment 28 days Chemotherapy** (virazole) 28 days Combined heat + virazole 28 days	+ - ++
Apple Cv. Ana, Rootstock Malus 70	CLSV	Heat treatment 28 days Chemotherapy (virazole) 28 days Combined heat + virazole 28 days	- + ++

*heat treatment includes exposure to 37-38C for 16 hrs light followed by 28-29C for 8 hrs dark.
**virazole 20 mg/L is included in the medium.
***evaluated as number of virus free regenerates. – ineffective; + effective; ++ very effective.

In Morocco, in vitro thermotherapy experiments are under way with the following almond cultivars/virus infected plantlets.
Tuono infected with PNRSV, PDV and PNRSV + PDV.
Ferraduel infected with PNRSV, PDV.
Fournat infected with PDV, PNRSV + PDV, PDV + CLSV.
Ferragnes infected with PNRSV + PDV.
Marcona infected with PDV, PNRSV + PDV.
Results will be summarized and compiled together with those obtained in Israel.

5. Adaptation of shoot-tip grafting (STG) for virus elimination (Morocco)

The STG method is based on excision of a small (less than 1mm) shoot-tip from infected plants (preferably grown in vitro) and placing it on a healthy, decapitated rootstock in vitro. Successful regeneration of the shoot tips allows growing new shoots, which may be virus free.

Since the last report, the work with STG was done with almond cultivars. Variations on the basic technique, aimed to simplify it and increase success rate have been studied. A significant improvement in this technique was achieved by using, surface sterilized, shoots from plants grown in the greenhouse rather than in vitro grown shoots.

Various plants were evaluated for their suitability to serve as 'rootstocks' for excised shoot tips. Results presented in Table 3 show that GF305 is the best rootstock among the tested ones and PM is second best. Surprisingly, GF677, a popular rootstock for field plants was inefficient for STG. The study of various parameters of STG will be continued and results will be compiled and prepared for publication.

Table 3: Effect of the rootstock on STG success

Rootstock	Variety	No of Plants	Successful grafts	Percentage	Total success in %
GF305	Marcona	20	17/20	85%	
	Ferraduel	20	11/20	55%	➢ 70%
	Tuono	20	14/20	70%	
	GF305	20	8/20	40%	40%
Pecher de Missour	Marcona	20	11/20	55%	
	Ferraduel	20	15/20	75%	➢ 53%
	Tuono	20	6/20	30%	
	GF305	20	11/20	55%	55%
GF677	Marcona	20	1/20	5%	5%
	GF305	20	10/20	50%	50%
Shirofugen	Marcona	20	1/20	5%	5%
	GF305	20	0/20	0%	0%
Myrobolan	Marcona	20	0/20	0%	0%
	GF305	20	1/20	5%	5%

C. Scientific Impact of Collaboration

Morocco

Ing. M. Zemzami (principal co-investigator) led ELISA assays and established the PCR technology lab at UCP. M. Zemzami is coordinating the project in Morocco. Ing. A. Rizqi took full responsibility of the tissue culture work involved in this project and is adapting the STG method for rosaceous hosts.

This project expanded the research activities of the UCP group in the PCR technology, in vitro propagation of rosaceous species, STG and other in vitro virus elimination aspects.

Israel

Dr. S. Spiegel is in charge of the tissue culture work including therapy, bioassays and ELISA. S. Spiegel is P.I. of this project. Dr. A. Rosner is responsible for molecular aspects. Dr. Zilkah is supervising the micro-propagation of indicators, rooting and hardening.

Exchange of technical information and know-how has become an integral part of this project. Several problems encountered by Moroccans in tissue culture and virus diagnosis were submitted to their counterpart scientists in Israel who suggested alternative approaches and/or improved methods. These include - vitrification, initiation of apple cultivars in vitro, ELISA for CLSV and thermotherapy of in vitro material. All these problems were either greatly improved or completely overcome.

Scientific links between collaborators in the two countries are now fully established and

contacts are being made on a weekly basis by means of electronic mail, fax and air-mail.

D. Description of Project Impact

The major impact of this CDR project is the interaction between Moroccan and Israeli scientists which resulted, among others, in the recent submission of a MERC proposal (M20-072) involving two labs in Morocco and two in Israel. Other immediate benefits resulting from this project are already obvious:

1. Strengthening virus diagnostic capability for rosaceons species in UCP lab.
2. Collection of valuable local almond selections from seedling populations in the Atlas Mountains.
3. Application of in vitro culture techniques to rosaceous species in Morocco. Initiation of apple cultures in vitro has been achieved for the first time in Morocco.
3. Application of in vitro culture techniques to rosaceous species in Morocco.
4. Development of the STG method (used in Rabat for Citrus) for rosaceous sp.

In Israel, PDV was identified for the first time in Israel in the frame of this project. PDV was detected in numerous sweet cherry cultivars by graft assays, ELISA and RT-PCR.

E. Strengthening of Developing Country Institutions

The Plant Control Unit (UCP) in Rabat, Morocco has improved through this project its capabilities in virus diagnostic and tissue culture techniques for rosaceous species. The tissue culture facility has been extended and a laminar flow hood and incubator for thermotherapy have been acquired. Recruiting a young scientist and technician labor provided by means of the project permitted the performance of extensive tissue culture and greenhouse work.

F. Future Work

With a few exceptions, the activities concerned with this project are now on schedule. It is expected to continue the research according to the initial work-plan.
Special attention will be given in Morocco to the vitrification problem in tissue culture, and the difficulties encountered in using ELISA for detection of CLSV. Now that initiation of apple in vitro has become less problematic in Morocco, our efforts will focus on virus elimination in apple by applying thermotherapy/shoot-tip culture to known, virus-infected, in vitro apple material. As mentioned previously, the STG method, an alternative virus elimination method which requires special expertise, is being studied extensively in Morocco by A. Rizqi who is highly qualified and experienced in this method in other plant species.

Section II

A. Managerial Issues

The project is on schedule in many aspects and behind schedule in thermo therapy and establishment of apple cultivars in Morocco. The latter is season dependent. In order to overcome these problems and fulfill the objective of establishing a reliable in vitro therapy technique for rosaceous fruit trees in Morocco, a request to prolong the project (without additional funds) to allow at least one additional spring seasons was requested.

B. Budget

No major changes were made to the budget.

C. Special Concerns

No protocols which address special concerns were developed.

D. Collaboration, Travel, Training and Publications

Close contacts are maintained between the collaborating labs by e-mail and fax. Attempts to further expand the collaboration resulted in a submission of a MERC proposal in August 98. Four- partner labs (both research and commercial) are involved - Morocco - M. Zemzami, M. Aaouine, Israel- S. Spiegel, E. Khayat. Ing. M. Zemzami and M. Aaouine visited Spiegel's lab in Feb. 99. The visit included discussions and future plans in the project, field trips to the south and north, a meeting with Mr. W.H. Crane and B. Ayalon of the US Embassy in Tel Aviv. Various technical problems encountered in Morocco regarding purchasing of supplies and transfer of funds were raised. A meeting of the four partners for the above pre-proposal took place in Rahan Meristems lab in kibutz Rosh Hanikra.

S. Spiegel visited Morocco in April 99. The progress of the project was evaluated with M. Zemzami, A. Rizqi and others and various problems were raised and discussed. Dr. Spiegel was later joined by three members of the Virology Dept (funds from other sources). A one-day meeting (open to the public) was organized in Rabat by UCP and DPVCTRF and hosted by the latter. Dr. Lakhdar (PDVCTRF) chaired the morning session. Ing. Zemzami presented an overview of the project and Drs. Spiegel, Raccah and Gera gave seminars on their current work. Ing. Zemzami translated from English to French. An active discussion followed the presentations. Attendance ranged from 65 – 150 persons during the day (program of seminars enclosed).

The April 99 visit in Morocco included also a trip to the almond seedling population in the high Atlas Mountains area to eye-witness the potential of this wealth.

Following the invitation to submit a full MERC proposal based on the pre-proposal, the four partners including Spiegel's, Zemzami's, Aaouine's, and Chayat, Duvdevani, got engaged in the preparation of the full MERC proposal. In mid-July 99, towards the final compilation of the MERC proposal, Ing. Zemzami and Dr. Aaouine (using funds from their own budgets) spent several intensive days with the Israeli partners in Rosh Hanikra, hosted by Rahan Meristem.

Ing. A. Rizqi spent 2 weeks in Spiegel's lab in late July, 99 and also attended the IPPC Congress in Jerusalem. Mr. Rizqi demonstrate the STG technique modified by him for stone fruit species and got 'hands-on' experience in tissue culture techniques in Spiegel's lab, as well as discussions with numerous scientists in the Institute of Plant Protection.

Publications

S. Spiegel, A. Rosner, Y. Tam, S. Zilkah, E. Faingersh, A. Rotbaum and L. Krizbai (1999). Detection of prune dwarf virus in sweet cherry in Israel. Acta Hortic. 472:249-255. Proceedings of the International symposium on Virus and Virus-Like Diseases of Temperate Crops. ed. A. Hadidi.

S. Spiegel, Y. Tam, A. Rosner, M. Brison, B. Helliot, A. Pierronnet, J.P. Eyquard and M.T. de Boucaud (1999).
In vitro elimination of prunus necrotic ringspot virus in a plum cultivar. In Plant Biotechnology and In vitro Biology in the 21st Century, pp. 545-546. Eds. A. Altman, M. Ziv, S. Izhar. Kluwer Academic Publishers.

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Typing Prunus necrotic ringspot virus isolates by serology and restriction endonuclease analysis of PCR products

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(*Accepted 30 July 1999*)

Summary

Prunus necrotic ringspot virus (PNRSV) isolates were characterised by bioassays, serotyping and restriction fragment length polymorphism (RFLP) analysis of PCR products. Based on symptoms in host trees and bioassays it was concluded that only one of the 16 tested isolates is severe. The serotyping results demonstrated that by using four different MAbs in TAS-ELISA the tested isolates could be divided into four subgroups, however, the severe isolate could not be singled out. RFLP analysis of PCR products supported the serotyping data but did not differentiate between isolates of the two main serological subgroups. A restriction map, derived from sequence analysis of the PCR products obtained from selected isolates, allowed exact location of the restriction sites within the PCR products of each isolate. A mild isolate with a unique genome structure was identified by both serological and RFLP assays. As far as we are aware, this is the first report on sub-grouping of PNRSV isolates by bioassays, serotyping with MAbs and RFLP analysis.

Key words: Prunus necrotic ringspot virus, ilarvirus, stone-fruits, serotyping, RFLP.

Introduction

Prunus necrotic ringspot virus (PNRSV), a pollen-spread member of the family *Bromoviridae*, genus *Ilarvirus*, occurs worldwide in *Prunus* species in numerous strains, varying widely in their pathogenic properties (Nemeth, 1986). Symptoms on PNRSV-infected stone-fruit species range from none or very mild to severe, including reductions in fruit yield and quality (Scott, Barnett & Burrows, 1989). Like other ilarviruses, the genome of PNRSV is composed of three RNA molecules (Fulton, 1983). RNA 3 codes for both the viral coat protein (CP) and a movement protein (Murphy *et al.*, 1995). PNRSV is of quarantine significance in many countries and, therefore, its detection in infected plant material is of prime importance. In addition to graft analysis performed traditionally with woody indicators, ELISA assays have been employed for detection of PNRSV mainly during the active growing season (Torrance & Dolby, 1984; Scott *et al.*, 1989). Recently, the polymerase chain reaction (PCR) technique was adapted for detection of PNRSV in infected peach trees (Spiegel *et al.*, 1996; Rosner, Maslenin & Spiegel, 1997).

Efforts to differentiate among PNRSV isolates were approached by several research groups. Mink, Howell, Cole & Regev (1987) produced polyclonal antibodies (PAbs) which distinguished three PNRSV serotypes (CH3, CH9 and CH30) isolated from sweet cherry trees showing symptoms of rugose mosaic disease. Crosslin & Mink (1992) demonstrated variations in the molecular weights of the CP of some PNRSV isolates. It was, therefore, assumed that the variability in biophysical and serological properties of these virus isolates may result from differences in the structure of their CPs.

The determination of the nucleotide sequence of RNA 3 for several PNRSV isolates (Hammond & Crosslin, 1995; Guo, Maiss, Adam & Casper, 1995; Sanchez-Navarro & Pallas, 1997; Scott, Zimmerman, Ge & MacKenzie, 1998) enabled differentiation among virus isolates based on direct analysis of their genomes. Primers derived from RNA 3 were used to amplify both the CP and 3A protein (movement protein) genes. Sequence comparisons among PNRSV isolates showed that both the movement protein and CP of PNRSV isolates are extensively conserved irrespective of either their original host or the geographic origin of the virus (Scott *et al.*, 1998).

In this study, we attempted to differentiate among diverse PNRSV isolates by applying serology, restriction fragment length polymorphism (RFLP) and bioassays.

Materials and Methods

Virus isolates and plant material

PNRSV isolates collected in Hungary and Israel from infected, local and imported, almond, apricot, peach, plum, a cherry rootstock variety and a rose bush were included in this study. PNRSV strains causing rugose mosaic disease (Mink *et al.*, 1987) were not identified in Hungary and Israel and, therefore, were not included. The geographical locations and symp-

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IN VITRO ELIMINATION OF PRUNUS NECROTIC RINGSPOT VIRUS IN A PLUM CULTIVAR

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Production of vegetatively propagated plant material is based on building up stocks from source plants Viruses entering a propagation system at the breeding or germ plasm level are transmitted vertically down the propagation chain . The ultimate outcome of this process is virus-infected planting material. Elimination of viruses at the beginning of the propagation chain will generally result in virus-free regenerated plants which can serve as a source for propagation. The most effective method currently used for elimination of viruses from plant material is a combination of thermotherapy followed by shoot-tip culture Chemotherapy has also been applied for virus elimination (Walkey, 1991) Following exposure to a high temperature or chemotherapeutant for several weeks, shoot-tips are excised from treated plants and either regenerated in vitro on a suitable culture medium into a new plant or grafted onto a virus-free plant. The virological status of the resulting plants is assesed several times over a given period of time during which virus-infected plants are discarded. The success of the three methods mentioned above, applied traditionally to developed plants grown in pots depends, among others, on the regenerability of small apices (1 mm or less), heat tolerance of treated plants and phytotoxicity of chemotherapeutants. Several woody species (e.g. *Prunus*) are very sensitive to heat and their exposure to elevated temperatures result in poor survival. Recently, cryopreservation was evaluated for it's potential to eliminate plum pox virus from a *Prunus* rootstock (Brison et al., 1997). This method involves chilling excised shoot-tips to -196°C in a protective solution and regrowing them in vitro on a suitable medium. Applying a virus elimination procedure to numerous apices in several hours rather than weeks may shorten the process considerably and is worthwhile evaluating for various host - virus systems.
Therapy procedures, increasingly applied to in vitro grown plant materials allow controlled modifications of media components and environmental factors which improve survival and regeneration of virus free apices (Stein et al., 1991; Spiegel et al., 1995).
In this study, in vitro grown shoots of plum (cv. Ogden) infected with prunus necrotic ringspot virus (PNRSV) were subjected to the following virus elimination treatments:

A. Altman et al. (eds.), Plant Biotechnology and In Vitro Biology in the 21st Century, 545–547
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November 1, 1999

Mr. William H. Crane
Science Counselor
U.S. Embassy
Tel Aviv

Ref: Grant No. TA-MOU-96-C16-059

Dear Mr. Crane

Please find enclosed copy of the progress report of the research work mentioned above.

Thank you for your cooperation.

Sincerely,

Riki Katzir

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