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Title of Project

EVALUATION, MASS-PROPAGATION AND CONSERVATION OF SUPERIOR, VIRUS-FREE PRUNUS LANDRACES

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

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

Project number: M20-072

USAID Grant Project Officer: Mr. L.J. Gumbiner

Project Duration: June 2001 – May 2005

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3. Executive Summary

The overall aim of this project is to advance the stone fruit growing industry in Morocco and Israel by facilitating production of certified propagation material of locally selected superior rootstocks and cultivars. To this end, the following objectives were proposed: (a) selection of almond genotypes from various regions of Morocco and evaluation for drought and salinity tolerance, (b) developing in vitro and /or in vivo rooting and propagation methods, (c) development of in vitro virus elimination methods for stone fruit rootstocks and cultivars, (d) validation of improved PCR techniques for detection of viral pathogens in post therapy in vitro shoot cultures, (e) optimization of protocols for mass micro-propagation of the selected clones (f) establishment of cryopreserved and in vitro maintained germplasm collections of selected Moroccan almond genotypes.

During the 1st year of the project the major activities were started, according to the planned work plan, both in Morocco and Israel.

- Superior almond clones (budwood and seeds) were collected by the Moroccan team from natural seedling populations in remote areas of drought and high salinity in Morocco. The prolonged recent drought decreased the seedling populations and therefore this activity will be extended to additional regions in the 2nd year.
- A collection of the selected clones, both grafted on rootstocks and initiated in vitro, was established and maintained in UCP.
- Collected seeds were divided between RM and UCP and germination was started for salt tolerance studies. Embryo cultures were also initiated.
- Preliminary salt tolerance studies with seedlings of bitter almond and Pecher de Missour (Moroccan rootstock) were started, following the protocol of RM.
- Stone fruit cultivars, of local interest in each country, were tested for PNRSV, PDV and CLSV and established in vitro for virus elimination and production of clean plants.
- Improved PNRSV detection and virus typing is being studied in Israel with a recently identified almond cultivar of high local significance, infected with a PNRSV isolate undetectable by ELISA.

The research teams communicate, exchange information, protocols and material on a regular basis and met for coordination meetings.

Section I

A. Research Objectives:

The overall goal is to advance the stone-fruit industry in Morocco and Israel by facilitating production of certified propagation material of locally selected rootstocks and cultivars. The specific scientific and technical objectives are:

a. Development of in vitro and/or in vivo methods for clonal propagation and rooting of mature almond idiotypes.
b. Selection of almond genotypes from various regions of Morocco and evaluation for drought and salinity tolerance.
c. Development and application of effective *in vitro* virus elimination methods for the targeted viruses. Adaptation and validation of sensitive detection methods for the targeted pathogens in post-therapy shoot cultures, aiming to shorten the production of virus-tested plants.
d. Adaptation and optimization of tissue culture protocols for mass propagation of the selected local plant material.
e. Development of a biochemical and/or histochemical assays for early evaluation of compatibility / incompatibility between scions and rootstocks.
f. Establish a cryopreserved / in vitro germplasm collection of almond genotypes.

B. Research accomplishments

The following activities were started during the first year of the project.

I. Morocco (UCP and EB)

1.1 Selection of superior clones of almond from traditional plantations of natural seedling in remote areas

Several visits were conducted by the Moroccan team to a number of localities in central, south and south-east Morocco. Vegetation in these areas suffered drastically from prolonged drought (see photographs in Appendix). Therefore, a limited number of selections were made as detailed in Table 1: Imintanout (15 selections), Tafraout (3 selections), Toundout (7 selections), Toundout valey (7 selections), Aguelmous (1 selection) and Nfifa (1 selection).

Collected material was established by grafting on Pêcher de Missour (PdM) in the greenhouse and was also cultured in vitro for initiation of shoot cultures. All collected material from the field was tested by ELISA for PNRSV, PDV and CLSV. Results are presented in Table 1.

Table 1: Collection of plant material from selected trees, initiation iv, grafting, sanitary status rejuvenation and seed harvesting

Selected Trees (collection sites)	In vitro initiation	Grafting / PdM	Sanitary Status			Rejuvenation	Seed harvest
			PNRSV	PDV	CLSV		
I1 (Imintanout)	+++	+++	-	-	-	+++	
I2 "	++	++	-	-	-	++	+
I3 "	+++	+++	-	-	-	+++	+
I4 "	+++	+++	-	-	-	+++	+
I5 "		++	-	-	-	++	+
I6 "	in progress	+++	-	-	-	+++	
I7 "	++	+++	-	-	-	+++	+
I8 "	++	+++	-	-	-	+++	+
I9 "	+	+++	-	-	-	+++	+
I10 "		+++	-	-	-	+++	+
I11 "	++	+++	-	-	-	+++	
I12 "		++	-	-	-	++	
I13 "		+	-	-	-	+	
I14 "	+++	+++	-	-		+++	
I15 "	+	+++	-	-	-	+++	
T1 (Tafraout)	+++	++	-	-	-	++	
T2	+++	++	-	-	-	++	
T3	+++	+++	-	-	-	+++	
T01 (Toundout)	+++	+	-	-	-	+	
T02 "	+++	+	-	-	-	+	
T03 "	++	+++	-	-	-	+++	
T04 "	+++	+++	-	-	-	+++	
T05 "	+++	+++	-	-	-	+++	
T06 "	++	++	-	-	-	++	
T07 "	++	++	-	-	-	++	
T0V1 Toundout Vallée)	+++	+	-	+	+	+	+
T0V2	in progress	+++	-	-	-	+++	+
T0V3	+++	+++	-	-	-	+++	+
T0V4	+++	+++	-	-	-	+++	+
T0V5	+++	+++	-	-	-	+++	+
T0V6	+++	+++	-	-	-	+++	+
T0V7	+++	+++	-	-	-	+++	+
Ag (Aglmous)	+++		-	-	-		
N (Nfifa)			-	-	-		

Names of collection sites are in prentices.

1.2 Collection of Almond seeds from selected trees
Trees from which seeds are collected are marked in Table 1. Seeds were divided between RM and EB for initiation of embryo-cultures. Agreements were made with owners of selected trees to preserve their production for our project at harvest. We returned to the visited localities to collect seeds several times to meet with some farmers that migrated from their home localities due to the ongoing drought.

1.3 Collection of imported almond varieties
Imported varieties of local significance were collected in almond plantings . These include: Ferraduel, Ferragnes, Fournat, Marcona, Tuono, Lauranne, Nec Plus Ultra, Mission, Nonpareil, Drak,Texas, Aï and Perles.

1.4 Collection of other stone fruit cultivars
Ten selections of apricot including Flavor Giant and local late selections were collected and established under greenhouse by grafting. One imported variety of plum 'Black Diamond' was also established in vitro and in vivo by grafting.

1.5 Collection of root-stocks
PdM, GF-677, a local selection of GF-677, Shirofugen, Myrobolan and 3 local seedling selections of Myrobolan were collected and established in vitro for mass propagation.

1.6 Collection of woody indicators
GF-305, PdM, Shirofugen, Pyronia, Virginia crab and Charden are established in vitro for multiplication for indexing needs.

1.7 Collection of virus infected varieties
A collection of almond varieties infected with PNRSV (1), PDV (2) and CLSV (3) are initiated in vitro for the planned sanitation experiments. These include Ferraduel (1), Fournat (1), Tuono (1), Ferragnes (1), Marcona (1), Fournat (1-2) and the selection TOV1 (2-3) (Table 1).

1.8 Rejuvenation Grafting
As planned in the activities of the project, all collected material from almond is established in the greenhouse. It is undergoing sequencial grafting on juvenile PdM seedling root-stocks for its rejuvenation. Some selections are at their second round grafting (Table 1).

1.9 Salinity tolerance test
GF-677 and PdM plants were evaluated for tolerance to salinity according to the protocol obtained from RM (detailed below). Three months old plants were irrigated with Hoagland solution supplemented with salt (NaCl) at increasing concentrations (from 25 to 400 mM). Wilting and chlorosis symptoms started to appear 4 days after the second irrigation with the highest concentration. This preliminary assay indicates that PdM is more tolerant than GF-677.

II. Israel (RM, VC)

2.1 The influence of salt on bitter almond plants (RM)

A preliminary experiment designed to evaluate the tolerance of almond seedlings to high salt was done with a population of bitter almond seedlings, traditionally used as rootstocks for almond in Israel.

The experiment: 124 test plants, grown in perlite, were irrigated with a ¼ strength Hoagland solution with an addition of various salt concentrations, while 10 control plants were irrigated with the ¼ strength Hoagland solution where no salt was added.

Irrigation was started with a 50 mM NaCl salt concentration and every 3 days 50 mM was added. After several days of irrigating with the 200 mM salt solution, discoloring of the leaves was noticed on many test plants and growth rate of those plants was lower than on the control plants (that didn't develop discoloring). During the experiment measurements were taken of the height of the plants as described in Table 2.

Table 2. Summary of average height of 200 plants at the different measurements

	Measurement 1	Measurement 2	Measurement 3
Average height of test plants (cm)	26.8	32	38.2
Average height of control plants (cm)	23.8	28.9	37.9

Table 3: Average height differences between the different measure dates.

	Control plants	Test plants
Average height difference between measurement 1 and 2 (cm)	5.1	5.2
Average height difference between measurement 2 and 3 (cm)	9	6.2

The growth rate of the test plants irrigated with salt containing solution was lower than that of the control plants, irrigated with the pure solution. Clones # 1,2,15,114 were observed to have more resistance to salt and they were chosen for tissue culture.

Table 4. Height differences of the more resistant plants at the measuring dates.

Plant number	Height difference between measurement 1 and 2 (in cm)	Height difference between measurement 2 and 3 (in cm)
1	6	10
2	5.5	10.5
15	9.5	13.5
14	7.5	13.5

The plants that were more resistant to salt were higher and greener than the other plants (Figure 1). It is clear that the growth rate of these plants was not influenced by the salt concentration and was comparable to that of the control plants.

Correspondingly, plants of clone GF 677 were irrigated with ¼ strength Hoagland solution containing 100 mM NaCl. They showed similar discoloring to that of the almond plants irrigated with 200 mM NaCl in the ¼ strength Hoagland solution. Two clones protruded as being more resistant to salt stress.

Figure 1

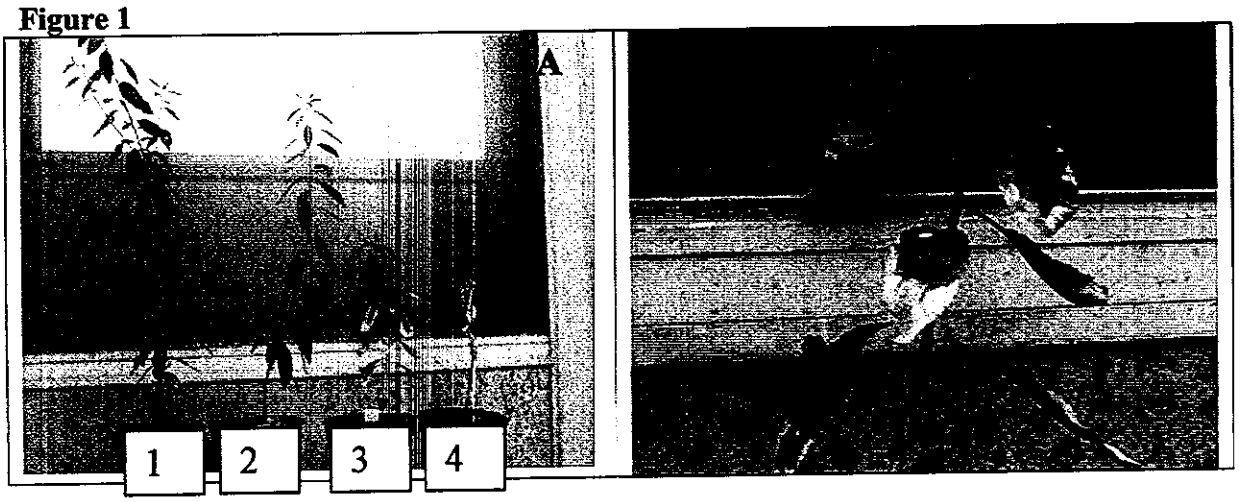


Figure 1A. Plant 1- Control. Plants 2-4 irrigated with salinity solutions (Plant 2 showing resistance). 1B. Salinity stress signs on almond plant.

2.2 Germination and salinity tests of PdM (RM)

Seeds of PdM were received from Morocco and germinated in pots filled with Perlite in a glass covered greenhouse. Samples were taken for virus testing directly in the seeds. The germinated plants will be tested in February 2003 for salinity resistance / tolerance as described above.

Concomitantly the clones of the bitter almonds tested in 2002 for salinity tolerance (described above) are grown under the same conditions and will be tested again for salinity resistance in 2003.

In addition, we received recently from our research partners in Morocco over 1000 seeds from the collected, selected trees (Table 1). Of the Moroccan collection we are currently germinating 500 seeds. The germinated plants will be tested in 2003 for salinity resistance. Selected clones will be preserved in vitro. Leaf samples from the germinated seedlings will be sent to the Plant Protection Service for virus testing to verify freedom from viruses.

2.3 In vitro culture of stone fruit rootstocks (RM)

Selected almond clones from the above experiment were placed in tissue culture. Several protocols were compared for stage I (explant initiation) and stage II (multiplication). As a comparison and for further experimentations, GF-677 and PdM were also placed in culture in the same growth media. The average multiplication rate of both almond and PdM ranged between 4-5. We currently have a stock of approximately 200 plants in vitro from PdM and 200 plants from each of 4 selected almond clones. The rest of the almond population is kept in vitro (3-4) plants of each.

Rooting experiments have already been initiated and will continue throughout the second year of the project.

2.4 Identification of stone fruit cultivars / selections of local or regional significance. (VC)
The stone fruit collection consisting of selections collected in Israel and local cultivars as well as imported stone fruit cultivars is maintained in Neve Yaar and supervised by Dr. Doron Holland. In the frame of this project almond, apricot and plums were tested for PNRSV, PDV and CLSV. The first test was done by ELISA assays in spring 2002. The test was repeated after about 2 months. RT-PCR assays were performed for samples with inconclusive ELISA results due to low virus levels (known in the ilarvirus group) and to verify ELISA results. This is the first evaluation of the virus status of this collection. Results are summarized in Table 5.

Table 5. Results of ELISA tests indicating the presence of PNRSV and PDV in the stone fruit collection in Neve Yaar (Israel).

Stone fruit crop	No. of cultivars / selections tested	Origin	No. of plants positive for	
			PNRSV	PDV
almond	14	Israel	4	0
apricot	13	Israel	1*	1?*
plum	6	Israel	2	
Total	**33**		**7 (21%)**	
almond	29	Imported	16	0
apricot	13	Imported	2	0
Total	**42**		**18 (43%)**	

*in one host.

PNRSV was quite common in both local and imported almonds, PDV was rare and CLSV was not detected by the ELISA assay. In the course of the work we discovered that detection of CLSV in almonds is problematic and not reliable both by ELISA and RT-PCR. Attempts to improve detection assays are underway.

The following cultivars and rootstocks were selected and established in vitro for virus elimination.
Almond 'Um El Fahem' (UEF) – a local selection made years ago, considered the most important variety in Israel. The trees in the collection did not exhibit disease symptoms.
Almond 54 (Kochav) – a local variety resulting from an Israeli breeding program in the 1960s (head Prof. P. Spiegel-Roy), a good variety and the major pollinator of UEF. The trees in the collection suffer from bare shoots and condensed growth, similar to 'bud failure' described in the literature.
Apricot Canino – the dominant, fresh fruit apricot in Israeli orchards. It is also of significant economic value in Morocco.
Rootstock 749 – a peach x almond hybrid. Considered a good rootstock and is of interest in Israel and Morocco. The plants in the collection are relatively young and appear normal.
Rootstock Pecher de Missour (PdM) – a peach x almond hybrid, a Moroccan native rootstock of high local significance, imported to Israel in the frame of this project.

UEF and 749 were found to be infected with PNRSV, detected easily by ELISA. Almond 54 was also found to be infected with PNRSV, however, the level of virus was consistently low, uneven distribution in the trees very high. ELISA was not reliable for detection. RT-PCR was reliable and verified the presence of PNRSV in ELISA negative shoots of 54. The level of PDV and PNRSV in apricot Canino were also low and results inconsistent. The presence of PNRSV in Canino leaves was verified by RT-PCR, however, a PDV specific band was not obvious.

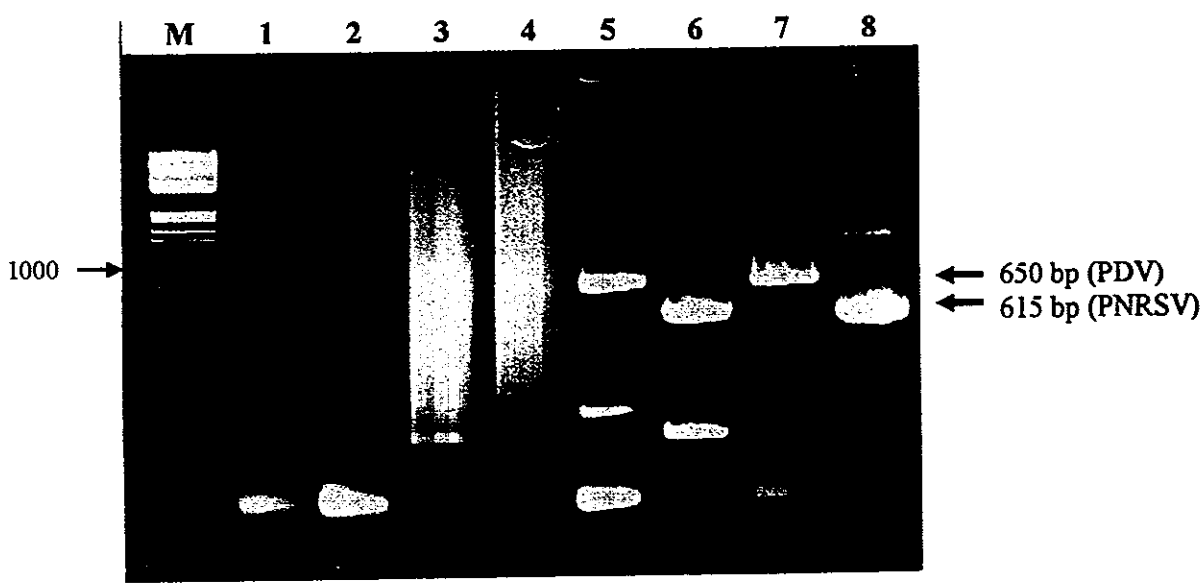
2.5 Establishment of in vitro cultures and virus status of clones (VC)

Budwood collected from field grown trees were used for establishment of in vitro shoot cultures, except for PdM which was obtained from young GH grown seedlings. In vitro shoot cultures were obtained using the standard procedures employed in our lab and were maintained on a clonal basis (one bud = one clone) on a Almehdi & Parfitt medium as described previously. Initially, apricot grew poorly in vitro. However, the addition of the hormone 6γγ dimethyl allyl amino purine (0.5 mg/liter medium), improved the growth of apricot in vitro significantly.

The presence of PNRSV, as determined by ELISA (BIOREBA reagents), was verified in all the resulting clones. Results were confirmed by PCR with specific primers. Due to the results obtained in the field with almond 54, about 40 clones were established in vitro and tested by ELISA. Out of tested clones, 39 were ELISA negative and only one clone was positive. Using a PCR assay and grouping 3-4 ELISA negative clones into single groups (total 10 groups) it was found that the ELISA negative samples were PCR positive.

The in vitro Canino clones were tested for PNRSV and PDV and were found negative (Fig. 2). The cultures are being maintained and retested before a decision will be made.

Figure 2. Detection of PNRSV and PDV in shoot cultures of apricot, cherry and almond grown in vitro.



M- DNA markers; (1, 2) apricot Canino, PDV primers; (3, 4) PNRSV primers; (5) Sweet cherry 40E50, PDV primers; (6) almond 54, PNRSV primers; (7) PDV plasmid; (8) PNRSV plasmid. Arrow indicates amplified specific PCR product.

2.6 In vitro thermotherapy treatments for virus elimination (VC)

Almond UEF, 54 and 749 were micro-propagated and thermotherapy trials were started.

UEF - survival through thermotherapy was very poor and no regenerates were recovered. An attempt to improve growth of this cultivar by adding the hormone 6γγ dimethyl allyl amino purine to the medium was begun recently. The thermotherapy treatment will be repeated.

54 – the ELISA–positive clone was micropropagated and two thermo - experiments were completed. Regenerates are being grown and will be tested soon by ELISA and PCR for virus presence / absence.

749 – currently being micropropagated.

The basic thermotherapy protocol used by us for stone fruit species is a cycle of alternating 38C for 16 hrs (light) and 28C for 8 hrs (dark). This treatment is applied for a minimum of 28 days to 72 shoots (2 shoots in each tube). For control, 20 shoots are kept in the growth room at 24C. Surviving shoot tips (main shoot and axillary buds) are excised (about 4 mm in length) and transferred to a growth medium. The first ELISA is done about 6 weeks after transfer and before the first subculture.

C. Scientific Impact of Collaboration

Morocco

Ing. M. Zemzami (principal co-investigator) is coordinating the project in Morocco and is the head of the UCP group. M. Zemzami was in charge of the expeditions to various parts of Morocco to identify and collect budwood and seeds of almond. Dr. Aaouine (until Aug 2002), Dr. Ait Chitt and Dr. Alamay participated in these expeditions. Dr. Alamy is in charge of the various tissue culture tasks and is doing the virus detection assays, supervised by Ing. Zemzami. Dr. Ait Chitt is in charge of the EB facility since Aug 2002.

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. The EB lab led by Dr. Ait Chitt, since August 2002, expanded its activities to accommodate the various tissue culture activities planned in this project for stone fruit species

Israel

Dr. S. Spiegel is P.I. of this project and is in charge of the tissue culture work, in vitro therapy and virus detection in VC. Dr. A. Rosner is responsible for molecular aspects.

Dr. E. Khayat is the group leader of the R&D lab of RM. He is in charge of the selection and screens for salinity tolerance of the rootstocks and varieties. Ms. Natalie Vinikur is a technical assistance both in the lab and in the greenhouse. Mr. A. Duvdevani, a tissue culture expert, is responsible for the TC activities in the project.

Scientific links between collaborators in the two countries are fully established and contacts are being made on a regular basis, mostly by electronic mail and fax. Exchange of technical information and plant material is an integral part of this project.

D. Description of Project Impact

The initial impact of this MERC project is the intensive joint work and interaction between the Moroccan and Israeli coworkers. Other immediate benefits resulting from this project are already obvious:

- Collection of valuable, endangered local almond selections (both budwood and seeds) from seedling populations in the Atlas Mountains and other remote areas in Morocco and establishment of a germplasm collection in Rabat / Meknes.
- Application of in vitro culture techniques to stone fruit species (focus on almonds) in Morocco.
- Focusing on studies aimed to develop salt tolerant rootstocks for stone fruit species.
- Strengthening virus diagnostic capability in UCP lab.
- In Israel, a local rootstock, widely used in Morocco was introduced in Israel and will be evaluated for the Israeli almonds.

E. Strengthening of Developing Country Institutions

Both UCP (Rabat / Salle) and EB (Meknes) have expanded their capacity and upgraded to accommodate the activities planned in the frame of this project. Morocco has improved through this project its capabilities in horticultural and tissue culture techniques, virus diagnosis and conservation of stone fruit species.

Recruiting scientific and technical personal, provided by means of the project permitted the performance of extensive planned work.

F. Future Work

It is expected to continue the research according to the initial work-plan as detailed in the proposal.

Several activities started in the 1st year will be continued in the 2nd year.

- The collection of almond selections from seedling populations in Morocco, planned for the 1st year (activity C.1), was not completed, due mainly to a severe drought in the target areas in south Morocco. This is a direct result of the ongoing lack of rainfall during the last 2-3 years. Therefore, the collection of vegetative plant material and seeds from almond seedling populations will be continued in the 2nd year and include additional remote areas.
- Seed germination and embryo culture was started towards the end of the 1st year and will be continued in the 2nd year.

Section II

A. Managerial Issues

The delay in transfer of initial funds caused a delay of the planned activities until January 2002. Since then the activities in Morocco are in progress according to the work plan.

Several personnel changes occurred during 2002.
- Dr. Mustapha Ait Chitt joined the Moroccan group (DDA) and Dr. Doron Holland the Israeli VC group. An approval for this addition was obtained from the US Embassy (TA).
- In summer 2002, Dr. M. Aaouine left DDA and returned to The Agronomic Institute in Agadir. Dr. M. Ait Chitt has taken over the whole operation in El Bassatine.

Necessary purchases needed to upgrade both UCP and EB labs were done according to the plan detailed in the proposal.

B. Budget

The Moroccan group is requesting a change in the list of equipment planned for years 2 & 3 without a change in the total budget. A letter to this affect is attached in the Appendix.

C. Special Concerns

No protocol which address special concerns were developed.

D. Collaboration, Travel, Training and Publications

- The interaction among the various research groups is very intensive. The Moroccan and Israeli scientists exchange frequent email messages, protocols, materials etc.

1st coordination meeting
- The kick-off coordination meeting took place in Barcelona (13-16 Feb. 2002) with the participation of the four groups leaders - M. Aaouine (EB); M. Zemzami (UCP); E. Khayat (RM) and S. Spiegel (VC). The meeting included intensive scientific and technical discussions, as well as managerial issues. The work plan for year 1 was reviewed and planned in great detail. In addition, a pre-arranged visit to the Institute of Agronomical Research (IRTA), Cabrils, Barcelona, took place. The Genetics group (Dr. Pere Arus, head) has been involved intensively in molecular and horticultural studies of *Prunus* sp. The information exchange with Dr. Arus and others was very beneficial to us.

2nd coordination meeting
- The coordination meeting summarizing the activities of 2002 took place in Bordeaux, France (9-14 Dec. 2002). The participants were: M. Zemzami and I. Alamy (UCP); M. Ait Chitt (EB); E. Khayat (RM); S. Spiegel and D. Holland (VC). The meeting was devoted to

reports of research activities presented by the group leaders, discussions of results, detailing the work plan for 2003 and addressing problems encountered during 2002. Assignments were divided re- exchange of protocols, samples etc.
The group spent a full day visiting INRA and Ctifl facilities (see Appendix). The visit in INRA Bordeaux included (1) the Virology lab, briefing by Dr. O. Le Gall, head of the lab (2) the Fruit Tree Research Station, discussion with Dr. F. Laigret, (director). The visit in Ctifl, Lanxade focused on a 'show and tell' of the in-greenhouse indexing for pome and stone fruit sp. in which this group specializes. Dr. P. Gentit (head, Virology group) spent several hours with us going through details and answering many questions.
A seminar entitled "Cryopreservation procedures for Prunus sp" was given to the group by Dr. M. de Boucaud of Bordeaux Univ.

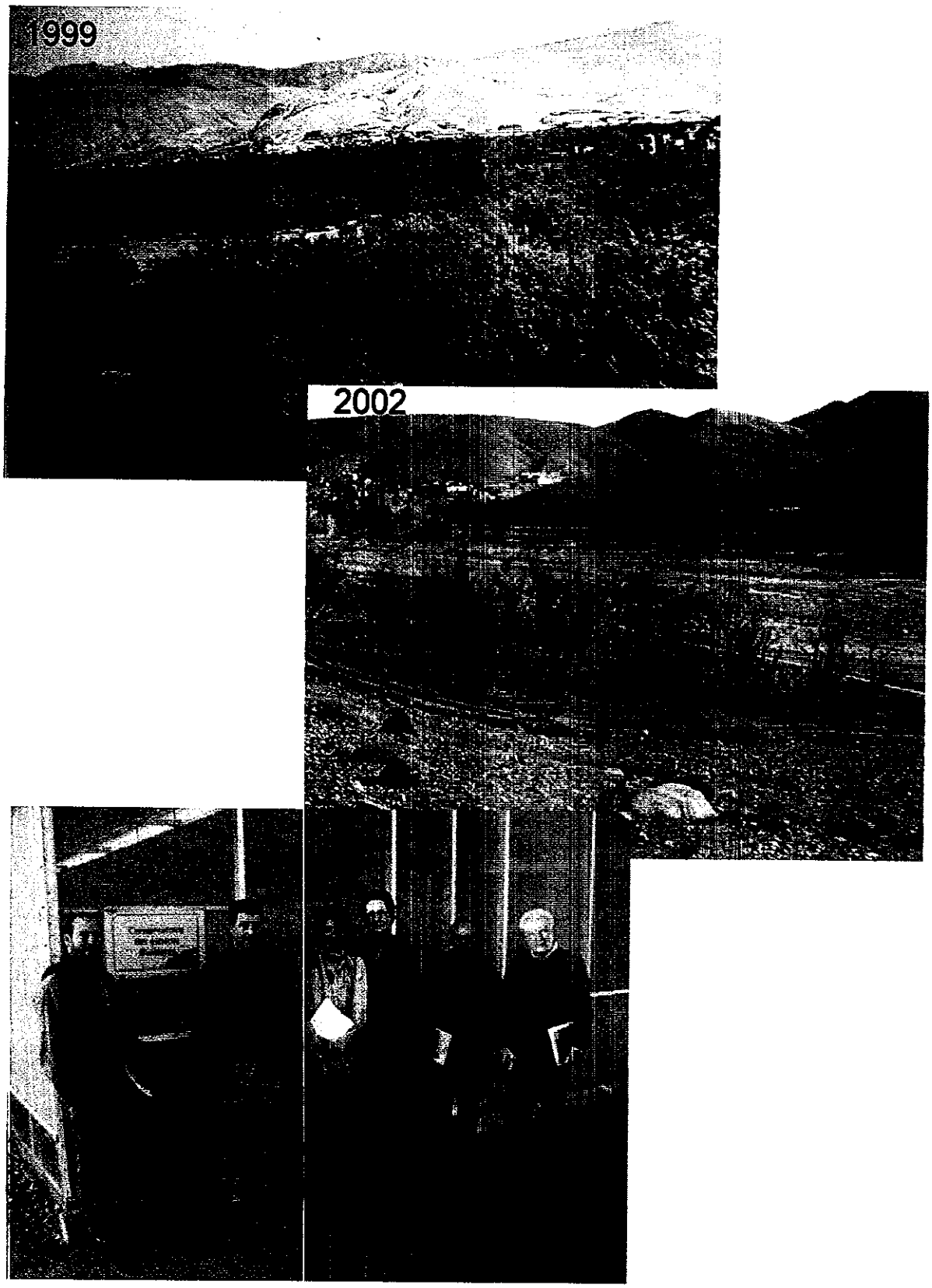
Collaboration with the Palestinian Authority
A collaboration regarding stone fruit viruses was established in the frame of this project between Spiegel's group and the UNESCO Biotechnology Education & Training Center (BETCEN), headed by Dr. N. Iraki, Bethlehem University. Mr. Omar Issa, a young scientist from BETCEN spent recently a training period in VC including Spiegel's lab. Publications, protocols and samples were made available to the trainee for his future work in BU. The parties agreed to strengthen the contacts in the future.

E. Request for American Embassy Tel Aviv or A.I.D. Actions.

We have concerns regarding the fulfillment of the objective aiming at training of young scientists in the frame of this project.
The current political atmosphere delays training of Moroccan young scientists in Israel and also in the USA. Therefore, we ask that an option of training in labs in European countries will be considered by the US Embassy Tel Aviv / A.I.D.

APPENDIX

The effect of prolonged drought on the almond seedling populations in South Morocco



Visit of the project co-workers in Ctifl, Lanxade, France (Dec 02). From left to right: M. Ait Chitt; M. Zemzami; I. Alamy; S. Spiegel; D. Holland; E. Khayat

DIRECTION DES DOMAINES AGRICOLES
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Date : Le 31 Décembre 2002

A l'Attention de : Dr Sara SPIEGEL, PI of MERC Project N° TA-MOU-00-M20-072

De la Part de : Monsieur ZEMZAMI, Responsable de l'Unité de Contrôle des Plants.

Réf. : 451 UCP/ZM/02

Objet : Request for a change in equipment items by El Bassatine (EB)

Dear Dr Spiegel,

I have received a request from Dr Mustapha AITCHITT (currently the head of the EB lab) to make a change in the list of equipment planed for EB within the scope of the above mentioned MERC project.
Dr AITCHITT argued that several items are badly needed and should replace other items which are available in nearby public institutions for which he has access. Therefore, Dr. AITCHITT is proposing the changes detailed below:

Items to be changed :

Year 2 :	
Microtome	$ 7,000.00
Microscope Slide Dryer	$ 1,200.00
Year 3 :	
Mino TM-Cryostate	$ 15,500.00
Total :	$ 23,700.00

Items needed :

Year 2 :	
Air-conditioning system (2 units)	$ 5,200.00
Sterilizing Oven	$ 3,000.00
Year 3 :	
Deep Freezer	$ 12,000.00
Refrigirator (2 units)	$ 2,400.00
Freezer	$ 1,100.00
Total :	$ 23,700.00

I hope it will be possible for Dr AITCHITT to have these changes which will help him accomplish the activities planned for EB in the frame of this project.

Sincerely, Mustapha ZEMZAMI