FINAL REPORT

Covering Period from: June 2002 – March 2006

Submitted to the U.S. Agency for International development; Bureau for Economic Growth, Agriculture, and Trade

TITLE OF PROJECT

Exploring the Tien-Shan Apricot Germplasm for Horticultural and Disease-Resistance traits

Award number: TA-MOU-01-CA21-011

Principle investigator: Dr. Sara Spiegel
Grantee Institution: The Volcani Center, Israel
Dept. of Virology, The Volcani Center, ARO, Bet Dagan 50250, Israel.
Tel: 972 3 9683561 Fax: 972 3 9604180 e-mail: spiegels@volcani.agri.gov.il

Co-Principle investigator #1: Dr. S.G. Dolgikh*
Investigator #2: Dr. G.P. Adrianova
Investigator #3: Dr. N.Yu. Nurtazima
Investigator #4: Dr. I.Yu. Kovalchuk
Institution: Kazakh Research Institute of Fruit Growing and Viticulture
Almaty, Kazakhstan.
Tel: 7 3272 482890 Fax: 7 3272 481050 e-mail: DolgikhSvet@mail.ru

Co-Principle investigator #5: Dr. D. Holland
Dept. of Fruit Culture, Newe-Ya'ar Research Center, ARO, P.O.B 1021, Ramat Yishay 30095, Israel. Tel: 972 4 9539528 Fax: 972 4 9836936 e-mail: vhollan@volcani.agri.gov.il

Administration Official: Dr. U. Mingelgrin
The Volcani Center
ARO, Bet Dagan 50250, Israel
Tel: 972 3 9683226; Fax: 972 3 9665327 e-mail: uriming@volcani.agri.gov.il

Administration Official: Dr. E. Madenov
Kazakh Research Institute of Fruit Growing
Almaty, Kazakhstan.
Tel: 7 3272 483202 Fax: 7 3272 633411

e-mail: spiegels@volcani.agri.gov.il

*since May 2004, ** retired
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C. Executive Summary

The overall goal of this project was to advance the stone fruit growing industry in Kazakhstan and Israel. The project focused mainly on apricot, since wild apricot populations exist in Kazakhstan. Traditionally, apricot has been grown in temperate regions of Central Asia. The following main activities were performed in this project.

Detection of PPV in Kazakhstan
A much needed indexing system for fruit tree viruses, based on grafting the woody indicator GF305, was established with budwood obtained from Israel. An ELISA system, including the necessary equipment, antibodies and other reagents, was set up in Almaty for virus testing. Observations for PPV symptoms and ELISA tests were performed in the Germplasm collection in the Pomological Garden and, where appropriate, in wild seedling populations. PPV symptoms were observed in local and imported plum and apricot cultivars but not in wild apricot populations. About 750 trees were tested for PPV by ELISA. A high percentage of samples from cultivars tested positive for PPV but not from the wild. The PPV isolate was identified as a D strain. Maps of the apricot and plum accessions in the collection with the PPV results were compiled. The maps demonstrate the dynamics of PPV spread from 1992 to 2005. Selected PPV isolates were transferred to Spiegel's group for partial characterization. Based on molecular assays, the identification of PPV strain D was confirmed. Further studies revealed a unique deletion of six nucleotides in the CP region, in several plum isolates. The PPV data generated in this project are new and contribute to the body of information on PPV.

Development of molecular tools for identification of PPV resistant apricot types
New technologies in apricot molecular genetics were developed in Holland's lab and transferred to Dolgikh's group. These included extraction of DNA from apricot leaves and DNA amplification and molecular fingerprinting. The methods enabled studying the variability between different apricot cultivars. This technology allows the use of molecular markers to assess virus resistance in apricot cultivars in Kazakhstan. An attempt to identify PPV resistant lines in the wild apricot populations in Kazakhstan was started. Seedlings, germinated from selected seeds of resistant forms of wild apricots in the South-east of Kazakhstan were established. Selected clones were assessed for resistance to PPV by a GF305 grafting test and by ELISA. Only 1/60 seedlings tested positive. After testing by PCR analysis the negative clones may be considered as candidates for PPV resistance.

Study of resistance to stress-factors under conditions of the south-east of Kazakhstan
The physiological values of productivity and stress - resistance of wild apricot plants taken from different ecological niches were determined. As a result of the investigations on wild fruit stands of apricot in the south and south-east of Kazakhstan the land belts with the least risk of repeated spring frosts and the most favorable for apricot growing were selected. Maps-schemes were compiled.

Visits and training
Three co-researchers from Kazakhstan visited Israel for exchange of scientific information, acquiring techniques (ELISA and genetic plant analysis). A young Kazakh scientist spent a 6 months training period in Dr. Holland's lab in Israel.
D. Research Objectives:

- Selection of apricot forms from wild populations for Plum pox virus (PPV) (Sharka) resistance / tolerance.
- Establish a detection bioassay system for PPV based on indexing and a large scale, serological test based on ELISA.
- Establishment of apricot population that segregates for resistance to PPV.
- Identification of genes involved in resistance to PPV in apricot.

E. Methods and Results

Kazakhstan

E.1 Problems of the apricot and plum growing industry in Kazakhstan

The majority of the world apricot mass is referred to the species Prunus Armeniaca vulgaris Lam. In the limits of this species, four main eco-geographical groups were distinguished: Middle-Asian, Iran-Caucasus, European and Dzhungar-Zailijskei. The latter group occupies the very northern part of the Middle-Asian area of the apricot natural habitat. This is the youngest sub-group of the Middle-Asian apricot group.

Under the conditions of the Almaty region, a considerable part of natural germplasm of apricot is concentrated. However, these populations are endangered. Wild apricot is registered in Red Book of Kazakhstan. The genetic diversity of the natural, wild apricot populations in the Tien-Shan mountain range in Kazakhstan offers an exceptionally important gene pool for breeding programs aimed at frost tolerance, and resistance to pathogens. Therefore, it is of great importance to maintain this valuable genetic material in a living germplasm collection. Photographs of apricot and plums in nature and in the collection in the Pomological Garden are presented in Fig. 1 in the Appendix.

The main natural factor, limiting apricot distribution in Kazakhstan is late-spring frosts. The periodical damage of flowers by spring frosts is characteristic for the whole territory of fruit zone of the Republic. Spring frosts cause mass death of apricot flowers that under conditions of Zailijskei Alatau leads to yield losses over 7-8 years. In 2004, no recurrent frosts were observed and the fruit from apricot varieties and selected wild forms was harvested. In 2005 after hot weather at the end of March (28°C) apricot began flowering but in April severe snow fall (-6°C) resulted in a freeze of apricot flower buds at the altitude of 700-1300 m above sea level.

The second factor, causing economic yield losses and death of apricot is PPV. Based on symptoms, PPV was identified in Kazakhstan since the 1980s. However, a survey using ELISA and observation of symptoms was conducted for the first time in the frame of this project (detailed below).

E.2 Apricot damages by frosts and Regionalization of the territory

The areas under cultivated apricot in south-east Kazakhstan are insignificant because of low winter-resistance of this culture. In 1940-50 the majority of plantations were the seedlings of domestic wild apricot, planted mainly in orchard-protected stripes. From cultivated varieties only selections of variety Krasnoshekiy prevailed. In those years at the Institute of Horticulture and Viticulture the testing of
Apricot was conducted on the winter hardiness of varieties: Tlor-Ziran, Krasnoshekiy, Dzhaupasak (middle Asian variety), Kzyl-Isfarak (middle Asian variety), Korolevskiy (France), Nikitskiy Krasnoshekiy (the Crimea), Luise (France), Makhtobi, Supkhany, Spitak, Kursadyk, Alexsander, Krasniy partisan (the Crimea) and Minsandzhali from Shreder (Uzbekistan). These varieties were planted in the collection plot of Kamenskoe Plato (Ponomarchuk, 1956). In 1984-1986 there was a mass import of apricot varieties from the former USSR countries. These varieties are currently maintained in the Pomological Garden.

Periodical flower damages by spring frosts are characteristic for all the fruit growing zones of Kazakhstan. The horticulture interests require the elaboration of the means of rational use of climate resources and also ways and means of enhancing plant adaptive potential, and increasing of the use of coefficient of the climatic resources at the expense of the optimization of the agro-technical growing methods. With this aim, ecological foundations of the rational apricot distribution for enhancing its productivity are elaborated. The culture demands and their supply by the environment factors according to phases, periods of vegetations and natural zones of habitats are considered, climatic and microclimatic peculiarities of the territory are evaluated, the regions of different favorable conditions for apricot growing are selected. In the regions of less favorable, but suitable for horticulture, agro-climatic regimes orchard management factors (agrochemistry, land planning, arrangement, scheme of planting, wind-protection etc.) are determined, aimed at enhancing plant productivity.

Apricot is a fruit culture with a very short dormancy period. The sensitivity for apricot frosts is noticed till the end of May. Year 2004 was favorable for apricot. The productivity of the varieties was in the range of 1 to 25 kg/tree, wild apricot forms - from 0 to 45 kg/tree. The low fruit yield of several varieties is connected with severe infection of PPV. In 2005 after hot weather in March (28°C), apricot began flowering and in the first week of April there was heavy snow (-6°C). All apricot flower buds at the altitude of 700-1300 m above sea level froze to death.

The analyses of severe winter recurrences and the late spring frosts showed that in the regions of industrial horticulture distribution in the south and the south-east of the Republic it is possible to distinguish the most favorable land belts for apricot growing. The most favorable lands for apricot growing in the south-east of Kazakhstan are situated in pre mountain-steppe zone at the absolute altitude of 600-800 m in Dzhambyl region, at 700-900 m of Almaty region; and in the south-east dry-steppe, partially desert-steppe pre mountain zone between absolute altitudes of 600-800 m. The best conditions according to frost recurrences are in the territories protected from cold air stream of the north and north-east. In Dzhambyl regions, these lands are situated in the south of Kurdaï region, in Almaty region-pre mountain part of Uigur, Chilik, Panfilov regions and partially east part of Enbekshi-Kazakh regions. On the base of analyses of winter conditions - the average of absolute minimum air temperature, possibility of critical temperatures, coefficient of winter severity, heat supply and frost-danger, we have conducted a regionalization of the Republic territory for horticulture.

Great extent of zones, the complexity of place relief and contrast of soil cover stipulate the wide diversity of growing conditions. In the limit of zones the regions with different favorable conditions for apricot growing are distinguished. Favorable zones according to the wintering conditions and recurrences of late-spring frosts for apricot growing are formed in 2 zones of horticulture: south-west pre-mountain desert-steppe and south-east pre-mountain dry-steppe.
As a result of observations on wild-fruit apricot in the south and south-east of Kazakhstan the most favorable land belts for apricot growing were distinguished. The maps-schemes were compiled. The zone territories according to wintering are divided into 4 sub-zones: 1-favorable; 2- satisfactory; 3- not quite satisfactory; 4- unsatisfactory conditions (details in progress reports).

E.3 Selection of apricot forms from wild populations for PPV resistance / tolerance

In 2004, resistant to frosts and symptomless forms of wild apricot were selected in Ketmen, the most favorable zone of wild apricot growing in the south and south-east of Kazakhstan. The seeds of these forms were collected, stored in the cold and later sown in the collection plot of the Pomological Garden. A population of 400 seedlings was obtained and grafted on GF-305 indicators. ELISA tests showed that only 1/60 tested samples was positive for PPV. Seedlings were selected from the resistant to stress-factors, without PPV symptoms. In spring 2005, during visual observation of grown indicators PPV symptoms were not found on apricot seedlings. The samples of these apricot seedlings were also tested by ELISA.

E.4 Diagnostics of PPV

PPV symptoms on apricot were first observed by us in 1990 on the varieties Nikitskiy Krasnoshchekiy, Korolevskiy, Spitak, Alexsander, Tlor-Ziran. Interestingly, symptoms on apricot are less obvious than on plum. Till 2004 apricot was not tested by ELISA in Kazakhstan.

In the frame of this project, a commercial ELISA kit (Durviz, Spain) was used for PPV detection. This kit has been used for surveys in USA, Canada and in Europe. DAS-ELISA tests with three PPV-specific MAbs - universal, PPV-D and PPV-M. Leaf samples were collected from several branches on each tree during the active growing season (June 2004, 2005) and stored over CaCl₂ at 4C for about 20 days, until completely dry. These samples were stored at 4C and analyzed by ELISA. In the survey we conducted, leaves from 300 variety-samples of cultivated and wild apricot forms and 268 samples of plum, were selected in 2004-2005 and tested for PPV using ELISA. We concluded that the distribution of PPV among cultivated apricot varieties in the Pomological Garden increased from 3% in 1986 to 54% in 2005, and among selected wild forms it was 20%. A total of 98 cultivated apricot samples (230 samples tested), 16 wild apricots (70 samples tested) reacted positively in ELISA with the PPV-specific universal MAb as the secondary antibody. Isolates of 40 samples of cultivated apricot were typed as PPV-D.

It was established that among cultivated varieties of apricot PPV infection reached 55%, among selected wild apricot forms – 20% and among Plum – almost 100%. These results show that one of the main factors limiting apricot productivity in Kazakhstan is PPV.

Maps schemes of apricot and plum collection orchards in the Pomological Garden showing the distribution of PPV from 1986 to 2005 were complied (Fig. 2 in Appendix). The apricot infection percentage is above 50% and plum infection almost 100%. In the apricot orchard of 1986 planting, more than 18% of trees died until 2005. In 1992 the PPV infection in this orchard was 25% and increased to 50% until 2005. In the plum orchard of 1987-1989 planting, more than 35% of trees died until 2005. In 1992 PPV infection was 80% and until 2005 the whole orchard was infected.
by PPV (Fig. 3). These maps will be used to study the spatial distribution of PPV in this location and establish recommendations for control measures.

Fig. 3. Dynamics of PPV distribution in the apricot and plum collection orchards in the Pomological garden.

![Dynamics of PPV distribution in apricot and plum collection orchards](image)

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**Israel**

E.5 Development of molecular tools for distinguishing between apricot types for the detection of PPV resistant types

The aim of this activity was to develop methodologies to distinguish between different apricot types and to use this information for the detection of virus resistant apricot cultivars.

Apricot viruses are a serious problem and in many developing countries were responsible for total destruction of the apricot industry. Therefore, identification of virus resistant apricot cultivars is of high importance for developing modern apricot industry. Several labs in the world are working in apricot viral resistance including the lab of Dr. A. Abbott in South Carolina. This group mapped the viral resistant genes in American and European apricot populations (Hurtado et al., 2002). The innovative
aspects of this project were to develop the capability to understand whether viral resistance in American and European apricot populations is similar to that of the Kazakh resistant cultivars. If not, it could lead to finding new viral resistant genes in the Kazakh populations. Such genes are of high importance because there are numerous potyviruses and the known resistance genes do not confer viral resistance to all of them.

In this project, a population of apricot seedlings was established in Newe Yaar. These seedlings were the result of directed genetic crosses between pollen from 'Orange red' on flowers of 'Canino' trees. The resulting seeds were stratified at 4C for three months and then sown in a plot in Newe Yaar. The emerging seedlings were replanted in an orchard which constitutes the population used in this project.

We have developed a rapid protocol for the isolation of DNA from apricot which is suitable for SSR analysis. For this purpose we used young leaves of apricot seedlings. The method is based on a combination of CTAB extraction with heat treatment. The DNA quality of this method is sufficient for SSR analyses. Using this method, we have isolated DNA from all the progenies of the population.

Recently, several labs have mapped PPV resistance QTLs in a few populations of peach (Dirlewanger et al., 2004). These data are of high importance since it allows us to examine whether the genes determining resistance in our Israeli and Kazakh apricot populations are similar, and whether they are located in the same locus. In order to answer this question we have synthesized SSR markers MDP-96-005 and SSR MDP-96-018. These SSR markers are localized relatively close to the strong PPV-resistance QTL allele mapped to the G1 linkage group in apricot, almond x peach and plum. Data obtained from the experiments are represented in Fig. 3 and Fig. 4. So far, we have analyzed 11 out of the 28 progenies of the population. We have separated the PCR products in two different ways. The first separation was done on 2.5% agarose gels and the second separation was done on a newly purchased device based on capillary matrix separation which offers a much higher resolution (Fig. 4). It is clearly seen that MDP-005 is polymorphic in our apricot population and that the seedlings are segregating in this population (Fig. 4). Since we do not have PPV in Israel, we could not yet perform resistance experiments to determine whether there is a linkage between the segregation of MDP-96-005 and PPV resistance. This could be achieved by sending scions from our population to Kazakhstan and analyzing their resistance to PPV in Kazakhstan. Unlike MDP-96-005, the other SSR marker MDP-96-018 is not polymorphic in our population and does not show pattern differences among the progenies (Fig. 5).

Fig. 4. SSR analysis with the polymorphic SSR MDP-96-005 for some of the apricot seedlings of the crossbred population. Virtual picture of samples separated on capillary matrix.
E.6 Detection and characterization of PPV isolates originating from Kazakhstan

One of the tasks of the Israeli Virology group was to assist the Almaty lab to establish a reliable indexing and ELISA detection systems for PPV in Kazakhstan. This goal was achieved and both systems have been operating and generating results (described above). The data on PPV in Kazakhstan is new and of interest locally and to the PPV research world community.

Molecular characterization of the PPV isolates from Kazakhstan was challenging. However, due to lack of facilities this work could not be done in Almaty. Since PPV was not yet found in Israel, working on this viral pathogen requires special authorization from the Plant Protection Services and is extremely limited. After overcoming import permit issues, a molecular study of PPV isolates from Kazakhstan has begun by Spiegel's group. The initial work was done during a 6 months sabbatical leave of S. Spiegel in Canada (with the group of Dr. Delano James, BC, Canada) and later was continued in Israel on a wider range of isolates, selected by Dolgikh and Spiegel and included, local, wild forms, imported and local cultivars.

The results of this study are detailed below:

- Detection of PPV in plum and apricot samples from Kazakhstan by RT-PCR, using specific primers to the CP coding region, confirmed the ELISA results.
- Strain typing techniques (sequence and RFLP) confirmed the identity of the PPV isolates from plum and apricot as a D strain and supported the results obtained by serological assays in Kazakhstan using MAbs. Sequencing of the Nib-CP region in the 5' terminus of the virus genome suggested that these isolates are not recombinants (Glása et al., 2004).
- Nucleotide sequence analysis of the entire coat protein coding region of a plum and an apricot isolate resulted in the identification of a unique deletion of six nucleotides in the N-terminal region of the plum isolate but not in the apricot isolate. This is the first deletion of this nature observed among PPV isolates. A paper reporting these findings was published (Spiegel et al., 2004).
- A more extensive study of selected nine plum and five apricot isolates was performed as described above. The sequence data obtained for these isolates showed that this deletion was found in 4/9 plums imported to the Pomological Garden in Kazakhstan about 20 years ago from former USSR countries and 0/6 apricot accessions. Sequencing results demonstrating these results are presented...
in Fig. 6. The obtained molecular and spatial distribution results will be studied together by Spiegel and Dolgikh in an attempt to determine (if possible) the source of the PPV isolate with the deletion. We intend to publish a joint paper in the near future.

Fig. 6. Multiple alignment of the nucleotide sequences of a selected region in the coat protein coding region of PPV showing the six nucleotides deletion.

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Samples include PPV isolates derived from plum (plum) and apricot (apr) in Kazakhstan. A PPV isolate derived from plum P5R8 in Kazakhstan (accession no. AY953261) (Spiegel et al., 2004) and a PPV-D isolate (AY953261) were also included. The letters in blue and the dashed line in black demonstrate the presence or the deletion of the 6 nucleotides (nt), respectively.

F. Impact, Relevance, and Technology Transfer

The apricot plantations in the territory of Kazakhstan suffer currently from spring frost and infection with PPV. Introduction of a selection process for the production of genetically resistant apricot forms to PPV and also late blooming traits will increase the productivity of this crop and make it profitable under local conditions. Moreover, the uniqueness of the apricot germplasm in Kazakhstan might reveal new PPV resistance genes. Since there are several PPV strains / isolates, it is very important to increase the extent of resistance genes into commercial apricot varieties that are suitable for growth in Kazakhstan. In this respect, identification of new PPV resistance genes will be a most valuable contribution to the control of this virus. The data obtained in this project is useful for typing the PPV resistant apricot varieties in Kazakhstan. Once typing will be completed it will be possible to analyze whether PPV resistant types could be found in Kazakhstan. If such cultivars will be spotted they will be used for the following purposes:
1. Genetic crosses for the production of PPV resistant apricot cultivars.
2. Molecular analysis and mapping of PPV resistant genes in the Kazakh apricot populations.
3. Identification of new PPV resistance genes.
4. Breeding for new and improved apricot cultivars.
The results will be used in all levels including laboratories, and practical agricultural aspects. The lab of Dr. Dolgikh could use the markers we found to survey wild apricot populations for PPV resistance, for mapping and for breeding. This could be accomplished by the new skills practiced in Israel by the trainee Ms. Natalya Pomelova.

The data generated in this project on PPV isolates originating from Kazakhstan, other Central Asia and former USSR countries are of great interest both at the practical and basic level. It is anticipated that control measures will be established in Kazakhstan.

G. Project Activities / Outputs

Meetings

In July, 2004 Dolgikh S. and Nurtazina N. and in September, 2005 Kovalchuk I. visited the Dept. of Virology, the Volcani Center, Bet Dagan, Israel and the Newe Yaar research center with the aim to get acquaintance with the experimental works of these centers. They had the scientific circulations in the laboratory of Dr. S. Spiegel, in Bet Dagan and Dr. D. Holland in Newe Yaar. Special attention was given to the experimental analysis on the determination of PPV by ELISA method. The consultations on the determination of DNA-resistance to PPV of apricot samples were conducted in the laboratory of Dr. D.Holland in Newe Yaar.

Publications


Abstracts of presentations in meetings


H. Project Productivity

A significant amount of work was performed in the frame of this project. A testing system for PPV (and other viruses) was established in Kazakhstan and a major testing for PPV was accomplished. Molecular characterization of selected PPV isolates was performed although not planned in the original workplan. An initial apricot population from the wild with a potential to find new selections was established. Molecular tools were prepared and shared with the group in Kazakhstan.
Fig. 2. Dynamics of PPV distribution in the plum collection in the Pomological Garden.
Several management problems rose during the work on this project. To mention a few: the import of chemicals, reagents (for ELISA) and plant material (GF305 for indexing) into Kazakhstan was a time consuming, long process. The Co-P.I. in Kazakhstan was replaced due to local reasons. The time required to teach our trainee the DNA and PCR methodologies was longer than predicted. There was a difficulty to identify a second trainee for a second 6 months period as originally planned. We planned to survey many SSR markers with our apricot population and actually surveyed only a few of them. We faced problems in finding the right conditions for isolating good quality DNA suitable for PCR work. In spite of these problems, both the Kazakh and Israeli co-workers feel that the collaboration was fruitful. The interaction between the groups in Almaty and Bet Dagan is on-going.

I. Future Work

We definitely think that the outcome of the cooperation in the frame of this project is establishing the foundation for future work. This work should include the continuation of screening the Kazakh apricot populations for PPV resistant types. This will be done by biological assays combined with the molecular techniques described above. The scientists in both countries intend to summarize virological data obtained recently for the PPV isolates from Kazakhstan and publish it jointly.

J. Literature Cited


Appendix

Fig. 1. Apricot and plum trees in Kazakhstan.
  a. Wild apricots flowering in Pomological Garden (April 06).
  c. Wild apricot near the river Turgen, 1200 m above sea level (April 2006).
  d. Late flowering wild apricot on mountain slope, at elevation 1600 m above sea level, (April 2006).

Photographs by Dr. S. Dolgikh