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**RAPID PROPAGATION OF VIRUS-TESTED
KAZAKH POTATO CULTIVARS
PROJECT TA-MOU-CA 16-018**

**ANNUAL REPORT FOR THE PERIOD
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BY

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- 1 -

| Table of Contents: | <u>page</u> |
|---|-------------|
| 1. Cover page | 1 |
| 2. Table of contents | 2 |
| 3. Cooperating scientists | 2 |
| 4. Executive summary | 3 |
| Section I | |
| 5. Research objectives | 4 |
| 6. Research accomplishments | 4 |
| 7. Scientific impact of collaboration | 6 |
| 8. Description of research impact | 6 |
| 9. Strengthening of developing country institutions | 7 |
| 10. Future work | 7 |
| Section II | |
| 11. Managerial issues | 8 |
| 12. Budget | 8 |
| 13. Special concerns | 8 |
| 14. Collaboration and publications | 8 |
| 15. American embassy | 8 |
| 16. Table 1 | 9 |
| 17. Table 2 | 10 |

Cooperating Scientists

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Y. Itzhak

Alexandra Levine

Dr. Emma Teverovsky

Executive Summary

The purpose of this project is to produce virus-tested tuber seed potatoes in Kazakhstan, as there is no certified seed production scheme in Kazakhstan. Because reinfection in the field is high, rapid propagation technologies were employed to obtain minitubers under insect protected conditions. These will be grown in the field for 2 generations before being supplied to the farmer as “elite” seeds. The following was done during this year:

1. About 50,000 tubers from virus-tested Nievsky plants were planted in Astana area in 1998.
2. During 1998 another batch of minitubers was obtained from the green- and screenhouses: 53,000 Nievsky and 48,000 Tamasha. In addition during 1998 another 4,800 plantlets of Nievsky and 6,300 plantlets of Tamasha were prepared and grown in tubes.
3. Potato virus S (PVS) and M (PVM) were purified in Almaty and antisera to them were prepared. Plants from Astana were tested against 6 viruses.
4. Plantlets raised from tissue cultures of various clones including Kazakh varieties produced minitubers, which were planted in pots to produce “seed” tubers – in Israel.

Planning and discussion of results between Kazakh and Israeli Scientist was done regularly by e-mail, and antisera from Israel were sent to Kazakhstan.

Section I

A. Research Objectives

The objective of this project is to create a pilot scheme for providing potato growers with virus-tested seed potatoes. This scheme is based on rapid propagation of virus-free base plants, obtained through meristem tip cultures, by micropropagation and minituber-production technologies.

The specific objectives for this year were:

To increase Nievsky tubers at the Nura farm and to obtain virus-free plants of cv. Tamasha. To continue to purify PVS and PVM in Almaty and to prepare antisera. Prepare additional virus-tested plantlets and minitubers in Astana and Israel and continue to test plants by serology in Almaty and Israel and continue to evaluate the riboprobe for PLRV in Israel.

B. Research Accomplishments

The work during this period was centered on the following:

a. The work in Astana (Akmola) – Dr. V. Shvidchenko

1. Production of virus-tested potatoes

In 1998 a yield of 28 tons of var. Nievsky was obtained at the NURA farm from one hectare. The planting material for this field were minitubers obtained from climatic rooms, glasshouses and screen (gauze) houses – where plantlets from the *in vitro* rapid propagation scheme were planted (See annual report from May 1998). Yields of Nievsky in commercial crops in this area were about 14 tons/hectare compared with 28 tons/hectare from the virus-tested minitubers.

During 1998 another batch of minitubers was obtained from the green- and screenhouses: 53000 Nievsky and 48000 Tamasha. These will be planted in 1999 in the field.

In addition during 1998 another 4800 plantlets of Nievsky and 6300 plantlets of Tamasha were prepared and grown in tubes. These were later planted in the climatic rooms and in the glasshouse.

Test for PVX, PVY, PVS, PVM and PLRV were done partly in Astana and partly in Almaty. Antisera were obtained from the Potato Institute in Moscow (Novikov B.N.) and those prepared in Almaty and in Israel (PVX, PVY, PVS and PVM). Antisera for PLRV were from a commercial Boehringer-Manheim kit. Results are summarized in Tables 1 and 2.

As seen from Table 1 the Nievsky plantlets in the tubes, climatic chamber and gauze screenhouse reacted negatively for all the 5 viruses tested. In the plants from the glasshouses and field low rates of PVY, PVS and PVX were observed. The presence of PVX is quite disturbing.

For Tamasha results were similar, but in addition PLRV was found in the field samples.

2. Surveys of viruses in weeds.

Presence of PVX, PVS, PVM and PVY was surveyed in three areas in Northern Kazakhstan: in the Makinskoe- Voznesenka area 230 km north of Astana (a wood-steppe zone), NURA Zelinograd area 40 km south of Astana (steppe zone) and on Bestjube Seletinsk 250 km east of Astana (steppe zone).

The following weeds were sampled and tested by the drop-precipitin test and by ELISA for PVX, PVY, PVS and PVY : *Amaranthus retroflexus*, *Chenopodium album*, *Sonchus arvensis*

b. The Work in Almaty- Dr. Alija Manadilova

1. Purification of viruses and preparation of antisera. PVS and PVM were purified from *Chenopodium amaranticolor* and tomato, respectively. For PVS 11.4 mg virus were obtained and for PVM – 4.6 mg. Purified virus preparations were checked by – SDS electrophoresis and were found to be free from plant proteins. After injection into rabbits about 60-75 ml of antiserum was obtained for each virus.
2. Samples from the potato plants from Astana were tested against PVX, PVY, PVS, PVM, PVA and PLRV by sandwich ELISA. Results are summarized in Tables 1 and 2. In Tamasha relatively high infection rates with PVM were observed. All plants tested negatively for PVA.

c. Production of plantlets *in vitro* in Israel. Dr. D. Levy

Plantlets of the Clones 3, 4, 5 of Nievsky and Clones mentioned in Table 5 of the previous report (April 98) were hardened in pots (10 cm in diameter) in potting mixture containing peat and vermiculite, in a greenhouse (20-25°C). The pots were moistened and covered with a transparent plastic sheet for 3-5 days without further watering.

About 90% of the plantlets were established and developed vigorous plants. After 60-80 days, small tubers (minitubers) were harvested, 4 -15 per pot depending mostly on the genotype and plant size. Of Nievsky, 65, 44 and 91 minitubers were harvested of clones 3, 4 and 5 respectively. Clone 4 was discarded due to weak development.

These small tubers (minitubers) were planted in 10 l pots for the production of 'seed' tubers. The plants were monitored during growth and the tubers

(after harvest) were stored. Dormancy was observed and after emergence the tubers were again planted in 10 1 for further multiplication.

The list of cultivars or clones grown in pot mixture in a 50 mesh screenhouse include: Nievsky (clones 3 and 5), Imit, Ori, Zohar, LTL and additional clones of *S. Phureja* and of various combinations of *Solanum* species.

d. Virus testing of Astana (Akmola) plants in Israel – G. Loebenstein

Plants from plantlets and minitubers (see annual report April 98, p. 7) were again tested serologically for PVX, PVY, PVS and PVM and for PLRV (with the DIG-labeled probe). All test were negative.

The Nievsky clones 3, 4 and 5 grown in Dr Levy's screenhouse were tested twice for PVX, PVY, PVS, PVM and PLRV by ELISA and gave negative reactions for these viruses.

Additional antisera for PVS and PVM were prepared. Some antisera were sent to Kazakhstan.

C. Scientific Impact of Collaboration

Participation and collaboration between the scientists involved was reasonable, though communications were not always satisfactory and various discussions were needed to persuade the participants to use modern virus testing methods. As a result of these discussions by e-mail a major improvement in diagnosis of viruses in Kazakhstan was achieved.

D. Description of Project Impact

An improvement in planning of experiments and diagnosis of viruses was achieved in Kazakhstan. Especially the ELISA lab at the Institute of Molecular Biology and Biochemistry in Almaty (Dr. A. Manadilova) is working extremely well both in diagnosis and preparation of local antisera of a high standard.

E. Strengthening of Developing Country Institutions

See D.

F. Future Work

The project is on schedule, but we would like to extend it, without additional funding till December 2000. This will enable us to obtain results from the harvest of 2000, yields and virus incidence.

The detailed plan for the coming year:

1. Evaluate the "elite" seed of Nievsky (28 tons) obtained during 1998 in farmers fields.
2. Planting at the "NURA" farm the minitubers of Nievsky (53000) and Tamasha (48000) produced during 1998 to produce "elite" seeds.
3. Continue to prepare plantlets of Nievsky and Tamasha from virus-tested source plants in tubes and later transfer them to climatic chambers, green- and screenhouses for production of minitubers.
4. Continue to test plant material from Astana in Almaty by ELISA.
5. Develop the DIG-labelled RNA probe in Almaty.
6. Continue the experiments with rapid propagation of Kazakh cultivars and their hardening in the greenhouse in Israel.
7. Produce minitubers to "seed" sized tubers in Israel and compare the Kazakh cultivars to other cultivars and monitor their health status.
8. Prepare additional antisera to supply the lab in Almaty,
9. Monitor by ELISA the health status of the Kazakh cultivars to be grown by Dr Levy.

Section II

A. Managerial Issues

No specific issues were encountered.

B. Budget

A request to increase the expenses for Materials and Supplies and to decrease the items for Traveling and Salaries was made and granted.

C. Special Concerns

No special concerns were encountered.

D. Collaboration, Travel, Training and Publications

Dr Alija Manadilova from the Institute of Molecular Biology and Biochemistry , Almaty, arrived on March 8 (1999) at the Institute of Field and Garden Crops Bet Dagan, for a period of 5 months and us involved in tissue culture research.

Publications:

A.M. Manadilova, G.G. Sadvakasova, E. Bekelman and G. Loebenstein 1998.

Diagnostics of potato virus Y by immunosorbent assay.

Biotechnologia -Teoria I Praktika 3: 50-54 (in Russian).

E. American Embssy.

Mr B. Ayalon from the U.S. embassy in Tel Aviv was most helpful in various administrative matters.

Table 1. Elisa tests of Nievsky (April 1998 – April 1999)

| Tube plants | | | Chamber of artif. climate | | | | Gauze | | Hothouse | | Field | |
|------------------|--|-----------|---------------------------|-----------|---------------------|-----------|--------------------|--------------------|------------|------------------------|------------|-----------|
| Time | 24/6/98 | 16/3/99 | 29/5/98 16/9/98 | | 20/8/98 21/12/98 | | 20/8/98 16/9/98 | 20/8/98 11/1/99 | | 20/8/98 10/9/98 | | |
| No. of passage | 11, 13, 15, 16, 17, 19, 20, 21, 25, 26 | | | | | | | Almaty | | Astana | | |
| Place of testing | Almaty | | Astana | | Almaty | | Almaty | Almaty | | Astana | | |
| Line | No. sample | Infection | No. sample | Infection | No. sample | Infection | No. sample | Infection | No. sample | Infection | No. sample | Infection |
| line 54A | 5 | 0 | - | - | - | - | - | - | - | - | - | - |
| line 8 | 8 | 0 | - | - | - | - | - | - | - | - | - | - |
| line 17 | 5 | 0 | - | - | - | - | - | - | - | - | - | - |
| line IV | 147 | 0 | 33 | 0 | 2 | 0 | 22 | 0 | 26 | 0 | 20 | PVX(1/20) |
| line V | 147 | 0 | 33 | 0 | 2 | 0 | 22 | 0 | 26 | PVY(3/26) PVS(1/26) | 15 | PVY(1/15) |
| line VI | 147 | 0 | 33 | 0 | 2 | 0 | 22 | 0 | 26 | 0 | 20 | PVY(1/20) |
| line VII | 147 | 0 | 33 | 0 | 2 | 0 | 22 | 0 | 26 | PVY(1/26) | 20 | PVY(1/20) |
| line XI | 147 | 0 | 33 | 0 | 2 | 0 | 22 | 0 | 26 | 0 | 44 | 0 |
| line XII | 147 | 0 | 33 | 0 | 2 | 0 | 22 | 0 | 26 | 0 | 44 | 0 |

1-6

Table 2. Elisa tests of Tamasha (April 1998 to April 1999)

| Tube plants | | | Chamber of artif. | | | | Climate | | Gauze | | Hothouse | | Field | |
|------------------|--------------------|-------------------------|-----------------------------|-----------|--------------------|-----------------|--------------------|-----------|--------------------|-----------|-------------------|------------|------------|-----------|
| Time | 24/6/98 | 20/8/98 | 10/6/98 16/9/98 | | 20/8/98 16/9/98 | | 10/7/98 16/9/98 | | 16/9/98 12/3/99 | | 5/6/98 10/9/98 | | | |
| No. of passage | 3 | | | | | | Almaty | | Almaty | | Almaty | | Astana | |
| Place of Testing | Almaty | | Astana | | Almaty | | Almaty | | Almaty | | Almaty | | Astana | |
| Line | No. sample | Infection | No. sample | Infection | No. sample | Infection | No. sample | Infection | No. sample | Infection | No. sample | Infection | No. sample | Infection |
| 1. line C-1 | 5 | PVY(1-5) PVS(1/5) | 33 | 0 | 2 | 0 | 17 | 0 | 20 | 0 | 15 | PLRV(1/15) | | |
| 2. line C-2 | 5 | 0 | 33 | 0 | 2 | 0 | 17 | 0 | 20 | 0 | 15 | 0 | | |
| 3. line C-4 | 10 | 0 | 33 | 0 | 2 | 0 | 17 | 0 | 20 | 0 | 15 | PVY(1/15) | | |
| 4. line C-5 | 5 | 0 | 33 | 0 | 2 | 0 | 17 | 0 | 20 | PVS(1/20) | 15 | PVY(1/15) | | |
| 5. line C-6 | 5 | 0 | 33 | 0 | 2 | 0 | 17 | 0 | 20 | 0 | 15 | PVY(1/15) | | |
| 6. line C-7 | 6 | 0 | 33 | 0 | 2 | 0 | 17 | 0 | 20 | 0 | 15 | 0 | | |
| 7. line 0 | 11 | PVM(11/11) PVS(2/11) | - | - | 2 | 0 | 17 | 0 | 20 | PVS(1/20) | 15 | 0 | | |
| 8. line 11 | 11 | PVM(8/11) PVS(2/11) | - | - | 21/12/98 | 0 | 17 | 0 | 20 | 0 | 15 | PVM(1/15) | | |
| Time | 29/9/98 -- 16/3/99 | | Passage: 5, 6, 7, 8, 11, 12 | | Line? | PLRV (12/15) | | | | | | | | |
| | No. sample | Infection | | | | | | | | | | | | |
| 1. line C-1 | 76 | 0 | | | | | | | | | | | | |
| 2. line C-2 | 76 | 0 | | | | | | | | | | | | |
| 3. line C-4 | 76 | PVX(1/76); PVS(1/76) | | | | | | | | | | | | |
| 4. line C-5 | 76 | 0 | 0 | | | | | | | | | | | |
| 5. line C-6 | 76 | 0 | 0 | | | | | | | | | | | |
| 6. line C-7 | 76 | 0 | 0 | | | | | | | | | | | |

102