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RAPID PROPAGATION OF VIRUS-TESTED KAZAKH POTATO CULTIVARS

PROJECT TA-MOU-CA 16-018

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BY

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Cooperating Scientist

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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. Field increase of virus-tested minitubers – Yields obtained were 26.55 tons for Nievsky (29.5 t/ha) and 20.72 tons for Tamasha (25.9 t/ha).

A crop of 197.7 tons of "elite" tubers of Nievsky (35.3 t/ha) was obtained in the Nura farm , but no crop was obtained from Tamasha.

2. Planting of about 9000 plantlets from Nievsky and 11000 plantlets from Tamasha.

3. ELISA testings from field and tube-plantlets for 5 viruses in Kazakhstan, showed relatively very low virus incidence.

4. Additional antisera were prepared in Kazakhstan and in Israel.

5. Two Nievsky clones were compared in Israel with two control cultivars.

Planning and discussion of results between Kazakh and Israeli Scientist was done regularly by e-mail, and antisera and the plasmid for the PLRV riboprope 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 the same as for the previous year:
To increase Nievsky and Tamasha tubers at the Nura farm and to obtain virus-free plants of cv. Nievsky and 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. Plantings under protected conditions (green- and screenhouses, and in controlled temperature chambers) for production of minitubers.
Nievsky: 9125 plants ; Tamasha: 11491 plants.

Samples were tested for PVX, PVY, PVM, PVS and PLRV partly in Astana and partly in Almaty. In Astana antisera from the Potato Institute in Moscow were used. For PLRV the Boehringer kit was used.
A total of 870 plantlets each of Nievsky and Tamasha were tested. In the tests in Astana none of the plants were found to be infected, while in parallel tests done in Almaty with their antisera in 3 Nievsky lines 10% of plants were found to be infected with PVY and one line with 10% of PVM . With Tamasha in one line 10% of the plants were found to be infected with PVY and PVM.

2. Field increase of virus-tested tubers.
Nievsky: 0.9 ha of minitubers and 5.6 ha "elite" seed tubers, yielding 26.55 tons (29.5t/ha) and 197.7 tons (35.3 t/ha), respectively. Commercial fields of Nievsky at the Nura farm were 14.3 t/ha.
Tamasha: 0.8 ha of minitubers, yielding 20.72 tons (25.9 t/ha). However, no crop of "elite" seeds was obtained (Tamasha is a new cultivar in this region).
Plants were tested for PVX, PVY, PVM, PVS and PLRV by ELISA in June, August and September 1999.

In Astana most of the times about 100 samples were analyzed. No infection was detected in the fields planted from minitubers during the first 2 sampling dates , while one Nievsky plant out of 500 from the "elite" seed tuber field, tested positive for PVY . In the tests done in Sept. 99 in the Nievsky field planted from minitubers 3 plants (out of 50 tested) reacted positively for PVY, and in the Tanasha field one plant (out of 48 tested) was positive for PVY.
Antisera were obtained from the Potato Institute in Moscow and for PLRV from a Boehringer kit.
Parallel tests done in Almaty in September 1999 with their antisera showed 25% infection with PVY and 12.5% infection with PLRV in one Nievsky line and 12.5% infection with PVY in another line of Nievsky – in first year field. Four other lines of Nievsky (in the first year field) tested negative for all the 5 viruses tested. Tamasha (first year field) – in one line 12.5% of the plants were infected with PVY, while 5 lines were negative for all the 5 viruses tested.
In an additional test done in December 1999 in Almaty on samples collected from first year field 10.7% and 21.4% of Nievsky and Tamasha plants, respectively, were found to be infected with PVY.

The discrepancy between the results of virus testing from Astana and Almaty could be due to the differences in antisera used (Moscow antisera and Almaty antisera – the later being more sensitive) and/or that the a major part of the ELISA tests in Astana were performed by non-professional students.

B. The work in Almaty- Dr. Alija Manadilova

The results of the ELISA tests done in Almaty were reported above.
8 ml globulins and 800µl conjugates for each of the following viruses was prepared : PVX, PVS, and PVM.
Dr. Alija Manadilova spent a training period in Dr. Levy's lab during March 8 - August 8, 1999, studying :1. Rapid propagstion of potatoes; and 2. Transformation of peppers (see attached report).

C. Production of Seed-tubers in Israel - Dr. D. Levy

Production of minitubers and seed tubers

Small tubers (minitubers) were planted after the termination of dormancy in 10 l pots for the production of 'seed' tubers. The minitubers of 2 clones of Nievsky

(clones 3 and 5) and few other cultivars were grown in 50 mesh screenhouse in pots in sterilized mixture to avoid soil diseases and infestation with viruses. 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 l pots in the screenhouse for further multiplication.

On June 1999, we harvested about 100 (7.700 kg) tubers from clone 3 and about 70 (3.050 kg) tubers of clone 5. The tubers were of various sizes, generally round shapped. The material was stored at about 6°C till early September, 1999 and then transferred for a few days to 15-18°C and then transported to Sha'ar Hanegev, an arid desert area, to be grown in the field in comparison to other clones and cultivars.

Field experiment

Field experiment was carried out in the autumn-winter of 1999-2000 at Sha'ar Hanegev in Kibbutz Or-Haner. The climate is semi-arid Mediterranean, with an average winter rainfall (October-March) of 240 mm. The soil is loess and fairly uniform to a depth of at least 100 cm. The field capacity is approximately 25% (volumetric content) and the 1.5 MPa moisture content 10%.

Seed tubers were planted on 17th of September 1999 in elevated ridget rows 96 cm apart with 4-5 tubers per row. Harvest took place on January 20, 2000.

A basic fertilizer dressing of 100 kg N and 160 kg P_2O_5/ha was applied before planting, and additional 200 kg N/ha was applied as top dressing during growth through the sprinkler irrigation system; disease were controlled by spraying of pesticides and fungicides according to the requirements.

The results of the field test are included in Table 1, where Nieski clones are compared to the control cultivars Hermes and Lady Rosetta.

Table 1. Field performance of Niewski (clones 3 and 5) as compared to control cultivars. Planted on 17.9.99 and harvested on 20.1.2000.

Cultivar	Haulm vigour	Resistance frost	Tuber Yield (kg/m²)	Dry Matter in tubers (%)	Sugar Content in tubers (%)	Tuber shape	Tuber Color	Flesh Color	Growth Cracks
Niewski (3)	good	good	1.8	17.3	0.25	round-oval	yellow	yellow	some
Nievski (5)	good	good	1.4	17.2	0	round	yellow	yellow	some
Hermes	good	intermediate	2.9	18.2	0	oval	yellow	yellow	none
Lady Rosetta	good	intermediate	1.9	18.5	0	round	red	yellow	none

D. Virus Studies in Israel

During this period additional antisera for PVX and PVY were prepared. The Dig-labeled RNA probe for PLRV was further evaluated in various samples of potato tubers and was found to detect the virus reliably.

A paper entitled "Rapid propagation of virus-tested potatoes in Kazakhstan" was presented at the 14th International Plant Protection Congress, Jerusalem, July 1999 (Abstract attached).

Various discussions with the partners and administrative matters of the project required quite a bit of attention.

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. There are still some variations between the results obtained in Astana to those in Almaty (see **B.a.)**

E. Strengthening of Developing Country Institutions

See **D.**

F. Future Work

The project will be extended without additional funding till December 2000. This will enable us to obtain results from the harvest of 2000, yields and virus incidence. Results from this year should give an evaluation regarding the possibilities of growing virus-tested seed potatoes by rapid propagation in Kazakhstan.

The detailed plan for the coming period:
1. Evaluate the "elite" seed of Nievsky (198 tons) obtained during 1999 in farmers fields.
2. Planting at the "NURA" farm the minitubers of Nievsky (9000) and Tamasha (11500) produced during 1999 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. Dr V. Shvidchenko intented to participate in the International Congress of Plant Protection in Jerusalem, but because of locust problems in his experimental fields in Kazakhstan, had to cancel his participation.

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).

V. Shvidchenko, A. Manadilova, G. Sadvakasova, L.F. Sozinova, D. Levy and G. Loebenstein 1999. Rapid propagation of virus-tested potatoes in Kazakhstan. 14th Intern. Cong. Plant Protection. p.87 (abstract).

E. American Embssy

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

Attachment 1

RAPID PROPAGATION OF VIRUS-TESTED POTATOES IN KAZAKHSTAN

V. Shvidchenko[1], A. Manadilova[2], G. Sadvakasova[2], L.F. Sozinova[1], D.Levy[3] and G.Loebenstein[3]
[1]Agricultural Institute of Astana, Kazakhstan; [2]Institute of Molecular Biology and Biochemistry, Almaty, Kazakhstan; [3]Agricultural Research Organization, Volcani Center, Bet Dagan, Israel

Potatoes are the second most important crop in Kazakhstan, grown on about 425,000ha. The average yield is only 8 ton/ha, less than 1/5 of the yields obtained in the U.S., Europe and Israel. "Seed" tubers are at present taken from the same fields grown for market potatoes. In 1992-3 using "certified seeds" from Holland yields of about 35-40 tons/ha were obtained.

Surveys for viruses in potato fields a high incidence of virus symptoms was observed. After establishing an ELISA laboratory in Almaty and preparing high titered antisera of PVX, PVY, PVS and PVM several surveys for viruses and aphids were performed in several regions of Kazakhstan. Local potato varieties were found to be highly infected (15-75%), most infections being due to PVX, followed by PVY and PLRV. Infection rates reaching 15-20% of PVS and PVM were also observed. Two areas with low aphid populations were located. In addition a highly non-radioactive Digoxinenin-labeled Riboprobe for detecting PLRV was developed. This probe detected PLRV in potato leaves and tubers at a limit of 1pg/ml compared to 2ng/ml by ELISA (Loebenstein *et al.* 1997).

Through meristem cultures and heat therapy virus-tested plants of the varieties Nievsky and Tamasha were prepared. These plants were propagated by tissue culturing and "minitubers" were prepared in A

Attachment 2

Report for Study Trip in Israel

By Dr Alija Manadilova
Institute of Molecular Biology and Biochemistry
Almaty, Kazakhstan

Period: March 8 – August 8, 1999.

Location: Institute of Field and Garden Crops, Agricultural Research Organisation, Bet Dagan, Israel. Supervisor: Dr, David Levy.

During this period I studied 2 subjects:

1. Rapid propagation of potatoes.
2. Transformation of peppers.

1. Potatoes.

Plantlets from Kazakhstan, Israel and US, free from viruses were propagated by subculturing 3 times in MS medium. In the first step about 100 cuttings were obtained from virus-tested base plants. The stem shoots, after removal of leaves, were sterilized (1 min in 70% ethanol, followed by 20 min in 20% CaOCl) , rinsed with sterilized water and placed in small plastic containers , containing agar plus Murashige and Skoog medium with addition of sucrose. All the work was done in laminar air-flow cabinets. It is expected to obtain within 5 months about 40,000 – 50,000 plantlets. These plantlets will be used for producing transplants, which can be transplanted directly to the field or for producing minitubers.

Techniques for excision of meristems from shoot tips was learned.

Furthermore I learned techniques to measure alkaloids in potato tubers. Contents of alkaloids and dry weight in different potato genotypes were compared.

2. Pepper transformation.

The work on pepper transformation is summarized in a separate page.

I also attended the 14th International Plant Protection Congress in Jerusalem and had extensive discussions with Prof. G. Loebenstein as to the results obtained within this project and possible cooperation and projects in the future.