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RAPID PROPAGATION OF VIRUS-TESTED KAZAKH POTATO CULTIVARS

BY

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Table of Contents: page

1. Cover page	1
2. Table of contents	2
3. Cooperating scientists	2
4. Executive summary	3
5. Research objectives	4
6. Methods and Results	4
7. Impact relevance and Technology Transfer	10
8. Project Activities/Output	10
9. Project Productivity	12
10. Future Work	12
11. Literature cited	12
12. Attachments	13
13. Tables 11 and 12	17

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3) Executive Summary

Potatoes are the second most important crop in Kazakhstan. Average yields are only 8 ton/ha, less than 1/5 of the yields obtained in the U.S., Europe and Israel. This is mainly due to virus diseases in the planting material. The purpose of this project was to establish in Kazakhstan a pilot scheme for producing virus-tested "foundation" and "elite" potato seed, as there is no certified seed production scheme in Kazakhstan. As re-infection in the field is high, rapid propagation technologies were employed to obtain minitubers under insect protected conditions. These were grown in the field for 2 generations before being supplied to the farmer as "elite" seeds.

An efficient laboratory using advanced tissue culture technologies, virus testing, propagation of minitubers, and field propagation of foundation and "elite" tubers became operational in Astana and by the end of the project (harvest of Sept. 2000) about 660 tons "elite" tubers were obtained. Checking for viruses during the last growing season (2000) by enzyme-linked immunosorbent assay (ELISA) showed only a low infection rate. of 2.5% by *Potato virus Y* in the 3rd year field-grown plants (seed potato class B), while no infection was observed in the younger generations. In commercial fields grown in the vicinity cumulative infection by viruses reached 15.5%. These "elite" seed gave an increase in yields of about 90% compared to commercial fields in the vicinity, i.e. 25.3 t/ha compared with 13.3 t/ha, respectively

In parallel at Almaty technologies for virus purification were improved and high tittered antisera were produced. A detection method of *Potato leafroll virus* in tubers with a nonradioactive RNA probe was established. The production of antisera and the diagnostic methods will be of further value to Kazakhstan.

Companies from neighboring countries- Kyrgistan and Russia showed an interest in this project and the Principal Investigator in Kazakhstan Dr. V. Shvidchenko moved to Krasnodar (Russia) in mid-February 2001, to establish a similar project there. This unfortunately will severely affect the continuation of the project in Kazakhstan.

4) Research Objectives

The objective of this project was to create a pilot scheme for providing potato growers in Kazakhstan with virus-tested seed potatoes.

Potatoes are the second most important crop in Kazakhstan. Average yields are only 8 ton/ha, less than 1/5 of the yields obtained in the U.S., Europe and Israel. This is mainly due to virus diseases in the planting material, as no certified virus-tested "seed " potatoes are available. Imports of certified "seed" potatoes from the Netherlands resulted in significantly higher yields, but the costs of these "seed" potatoes were prohibitive to the local farmer.

The purpose of this project was to establish in Kazakhstan a pilot scheme for producing virus-tested "foundation" and "elite" potato seed, as there is no certified seed production scheme in Kazakhstan. As re-infection in the field is high, rapid propagation of virus-free base plants, obtained through meristem tip cultures, by micropropagation and minituber- production technologies. These were grown in the field for 2 generations before being supplied to the farmer as "elite" seeds.

This approach of "Rapid Propagation" or "Reduced Generation Scheme" for virus-tested potato tuber seed production is now a new developing area (Maene, 1994; Watad *et al.*,2001), suitable for the rapid build-up of certified potato "seed" supply. Such schemes are in operation in Israel, South Africa and by American Ag-Tech in the US (Watad *et al.*, 2001).

5) Methods and Results

This project was carried out in cooperation between 4 research groups: The Agricultural Institute in Astana (Akmola) (Dr. V. Shvidchenko) – Tissue culture and field propagation; Institute of Molecular Biology, Almaty (Dr. A. Manadilova) – Virus purification, preparation of antisera and virus testing; Institute of Field Crops, ARO, Bet Dagan (Dr. D. Levy) –Tissue culture of Kazakh potato cultivars and their evaluation; and Dept. of Virology, ARO, Bet Dagan (Prof. G. Loebenstein)- Preparation of Antisera, Improving detection methods, DIG- riboprobe , coordinating the project.

5.1. Methods for obtaining virus-free plantlets from meristems and preparation of minitubers - Akmola

The main variety propagated in vitro was Nievsky. Niewsky plants were grown at 37-38C for 5-8 weeks for thermotherapy, after which meristems were taken. Tests were made to increase the amount of sucrose in the nutrient medium (Murachige and Skoog, MS). Results are summarized in Table 1.

Table 1. Propagation of var Nievsky on MS medium with various contents of Sucrose (1997).

% sucrose	No. of shoots/plant	No. of days on MS till formation of plantlets	No. of roots	Viability of plants after transfer to soil %
1%	8	22	3	40
2%	10	26	3	50
4%	14	32	5	80

Best results were obtained with 4% sucrose in the nutrient medium. The number of shoots was highest; plants had a more developed root system, plants looked well, though some delay in development of plantlets in the tubes was noted. As the overall performance on 4% was better this combination was subsequently used.

From the tubes plantlets were transferred to pots or boxes with a soil mix and from they're to gauze-protected screenhouses (during the spring-summer season) or to climatic chambers or greenhouses (during the autumn - winter season). Transplanting the plantlets from the tube directly without transferring them first to pots or boxes for hardening caused a 40-60% less of the plants. Best results were observed in the gauze-protected screenhouses during the spring-summer season (Table 2).

Table 2. Results of plantings during Spring-Summer and Autumn - Winter Seasons (1997).

Season and growth conditions	Distance between plants	No. of tubes from one bush	Yield of tubers from 1000 plantlets	
			No.	grams
Autumn - Winter Nura farm greenhouse	20 X 50	7.4	267	7,397
Spring - Summer Gauze screenhouse	20 X 50	20.3	428	20,300

Apparently growing conditions during the spring-summer season in the gauze screenhouse were much better than during the autumn-winter season.
Cuttings from tubes were also propagated in a temperature controlled growth chamber.
Planting distance was 12 x 12 cm. Tubers obtained ranged between 0.5 - 1 gr. minimum to 10-12 gr maximum. The average number of tubers was 12 per plant.
The total number of tubers obtained was 15.300 from the gauze screenhouse (spring-summer) 12.000 from the glasshouse (autumn-winter)
23.000 from the temperature chamber (spring-summer and autumn - winter) altogether
50.300 tubers of Nievski. Plants were tested for virus incidence: 2000 plants from the winter glasshouse, 1000 from the gauze screenhouse and 3000 from the growth chamber. For ELISA testing antisera prepared by the Russian Korenevo Potato Research Institute in Moscow were used. A few plants (less than 10) were found to be virus infected and were eliminated. However, in comparative tests with the diagnostic kits from Almaty they were found to be more sensitive than the Moscow kits.

Virus incidence in commercial field grown potatoes from Akmola in summer 1997 were tested by ELISA in Almaty. Results are presented in Table 3.

Table 3. Virus testing of field grown potatoes from Akmola (1997).

Variety	Date	No. samples	PVX %	PVY %	PVS %	PVM %	PLRV %
Nievsky	6.7.97	13	15.3	0.0	7.69	0.0	23.07
Nievsky	7.7.97	9	33.07	0.0	0.0	0.0	46.15
Nievsky	7.7.97	23	8.69	4.39	8.69	0.0	17.39

As seen from Table 3 commercial fields in Akmola were heavily infected by PVX and PLRV.

5.2 Total number of Minitubers prepared during the Project - Astana

The amount of minitubers prepared during the project is summarized in Table 4.

Table 4. Number of Minitubers prepared during 1997- 2000

Year	Niewsky	Tamasha
1997	50,000	
1998	53,000	48,000
1999	9.000	11,000

5.3. Virus testing of Astana's plantlets in Almaty

Virus tested nuclear plants of 2 local varieties: var. Niewski (second year, 50th passage, three lines) and Tamasha (first year, 7 lines, 4th passage) were prepared by meristem - tip culture in the Agricultural Inst. of Akmola.

Testing was done by ELISA against PVX, PVY, PVS, PVM and PLRV.

Antisera - globulins conjugates to PVX, PVY, PVS were prepared in Kazakhstan: PVM - in Israel, PLRV from kit (Boehringer Mannheim).

Results from 1998 are summarized in Table 5.

Table 5. Results of ELISA testing in Almaty (1998).

Variety	Date	No. of samples	PVX %	PVY %	PVS %	PVM %	PLRV %
Nievsky	6/2/98	3	0	0	0	0	0
Tamasha	4.2.98	7	0	0	0	100	0

The Tamasha plants were discarded and another attempt was made to prepare nuclear virus-free plants from meristems after heat treatment of the Tamasha source plants.

In 1999 additional batches of Niewsky and Tamasha plants from meristems were tested for the 5 viruses mentioned above and PVA. A total of 870 plantlets of each Niewsky and Tamasha were sampled. In 3 lines of Niewsky 10 % of the plants tested positive for PVY and in one line 10% of the plants carried PVM. With Tamasha one line was infected by both PVY and PVM. Lines, which were infected, were discarded.

In 2000 additional batches of Niewsky and Tamasha plants from meristems were tested for PVX,PVY, PVS, PVM and PLRV. A total of 60 plantlets of each Niewsky and Tamasha were sampled. All Niewsky plantlets tested negative for all the five viruses, while for Tamasha one line reacted positive for PVY(10%) and PLRV (10%) and in a second line 10% of the plants were positive for PLRV.

5.4. *Field increase of Minitubers and "Elte" seed in Astana*

Minitubers were planted in a field at the "Nura" farm near Astana. Plantings of minitubers are summarized in Table 6.

Table 6. Size of fields planted with minitubers at the "Nura" farm

Planting Period	Niewsky	Yield (t/ha)	Total Yield // (tones)	Tamasha	Yield (t/ha)	Total Yield (tones)
1998	1 ha	28	28			
1999	0.9 ha	29.5	26.55	0.8 ha	25.9	20.7
2000	3 ha	22.8	68.3			

Plantings of first-and second-year Niewsky potatoes at the "Nura" farm are summarized in Table 7.

Table 7. Size of fields planted with first-and second-year Niewsky potatoes at the "Nura" farm and their yields.

Year	First-year potatoes			Second-year potatoes		
	Acerage	Yield (t/ha)	Total Yield (Ton)	Acerage	Yield (t/ha)	Total yield (Ton)
1999	5.6 ha	36.3	203			
2000	4 ha	26.1	104	30 ha*	24.47	741

* Including plots at 2 additional sites (University and "Plant of Seed" farm).

Commercial yields of Niewsky in the Astana region during the time of the project are presented in Table 8.

Table 8. Commercial yields (t/ha) of Niewsky potatoes in the Astana region.

Year	Yield (t/ha)
1998	14
1999	14.3
2000	11.5

Comparing yields between the "elite" tubers from the project (Second year field-grown potatoes) - 24.47 t/ha , with yields of commercial fields – average from 3 years- 13.3 t/ha showed an increase in yields of about 84%.

5.5 *Virus-testing of Potato Fields planted with Minitubers , First- and Second-year Niewsky Seed Potatoes*

Tests were done by ELISA both in Astana and in Almaty. Results of the tests done in Almaty are presented in Table 9. The results of tests done in Astana were generally much lower than those from Almaty. This discrepancy could be due to the difference in antisera used (Moscow antisera and Almaty antisera – the later being more sensitive) and/or that the major part of the ELISA tests in Astana were performed by non-professional students.

Table 9. Virus infection (%) in potato fields planted with minitubers, first- and second-year Niewsky seed in Astana. ELISA tests were done in Almaty

Year		PVY	PVX	PVS	PVM	PLRV
Minitubers	1998	5.5	1.3		1.1	1.1
Minitubers	1999	5.1	0	0	0	1.3
Minitubers	2000	0	0	0	0	0
Furst-year	1999	10.7				2.1
Second- year	1999	2.0			2.0	
First-year	2000	0	0	0	0	0
Second-year	2000	2.5	0	0	0	0
Commercial Field	2000	0	2.8	4.2	8.5	0

As seen from Table 9 the seed tubers produced within this scheme have a relatively low percentage of virus infected plants, better than the Dutch seed potatoes. It should also be noted that there is a gradual decrease of virus infection over the years, probably due to the experience gained over the years.

5.6 Purification of viruses and preparation of antisera-Almaty

Viruses PVX and PVY were purified in Otar from leaves of *Nicotiana tabacum* Samsun (NN) and *Datura stramonium* which were grown in a greenhouse (in our laboratory in Almaty). Purified virus after checking by gel electrophoresis (PAGE) was injected into rabbits for antisera production.

30 ml antiserum to PVY and 35 ml - to PVX were obtained. 10 ml - globulins and 600 microl. conjugates for every virus, were prepared.

Virus PVS was purified by the modified method (Sadvakasova, Bekelman, Loebenstein 1997) from leaves of *Chenopodium amaranticolor.* Purification PVM was done according to a protocol, obtained from Israel (See Attachement 1).

From 220 g of infected leaves of *Chenopodium amaranticolor* 1.220 mg PVS were obtained.

From 138 g. of infected tomato leaves 0.68 mg PVM were obtained (Apparently the concentration of virus in tomatoes leaves was low).

5.7 *Adaptation of a riboprobe for testing PLRV- Almaty*

Attempts were made to adapt the riboprobe in Almaty. This was only partially successful as in several test no PLRV-infected controls were available and new membranes had to be obtained (from Israel). The tests will be continued and could become a standard method for detection of PLRV in non-sprouted tubers.

5.8. Production of plantlets in vitro - in Israel

Tissue cultures of numerous potato clones were established in vitro. These include Solanum tuberosum cultivars, other tetraploid clones, and other genetic combinations.

Shoot cultures were originally established from actively growing shoots of greenhouse-grown pathogen-tested plants. After leaf removal and shoot sterilization (1 min in 70% ethanol followed by 20 min in 2% $CaOCl_2$, and a rinse with sterilized water), stem segments containing one node were placed in 13 x 100 mm glass tubes. The tubes contained 1.5 ml of MS with 12 g l^{-1} agar (Agar-agar, Sigma Chemical Co., St., Louis, MO, U.S.A), 30 g l^{-1} sucrose, but no hormones. The tubes were sealed with transparent 13-mm polypropylene tube closures and placed in day/night temperatures of 26/20°C. Illumination was provided by 40 W fluorescent "cool white" lamps, providing a light

intensity of 316 μW $(cm^2)^{-1}$. The illumination period was 16 h. Aseptic procedures were all carried out in a Gelaire laminar air-flow cabinet with an air velocity of 27 m min^{-1}. New shoots developing from axillary buds were sub-cultured by cutting single-node segments and placing each segment on fresh medium. After several sub-cultures, uniform shoot growth and multiplication (5-7 segments per 3-4 weeks) were established.
Few of the genotypes are mentioned in Table 5.

Table 10. Potato clones established in vitro.

Species*	Genotype	Origin
tbr	cv. Desiree	Netherlands
tbr	cv. Spunta	Netherlands
tbr	cv. Kennebec	U.S.A.
tbr	cv. Norschip	U.S.A.
tbr	cv. Baronesa	Brazil
tbr	cv. Huinkul	Argentina
tbr, dms	cv. Cruza	Mexico
tbr, adg, dms	cv. Rosita	Mexico
tbr, phu, dms	BR-63.15	U.S.A.
tbr, adg, dms, sto	B-71-240.2	Argentina
tbr	Nievski	Kazakhstan

*tbr, S. tuberosum; dms, S. demissium; sto, S. stoloniferum;
adg, S. andigena; phu, S. phureja.

5.9 Hardening in the greenhouse
Plantlets of these genotypes were taken out of the flasks and planted in vivo in small 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 Nievsky, 65, 44 and 91 minitubers were harvested of clones 3, 4 and 5 respectively. Clone 4 was discarded due to weedy development.

5.10 ***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 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.

5.11 Field experiment: Winter of 1999-2000 (Table 11)
The 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

Yields of Nievsky clones were average. Due to their round shape they were suitable for chip processing. However, clone 3 have had relatively high sugar content which caused nondesirable browning of the chips (Figure I).

5.12 Assessment of heat tolerance

A range of cultivars and clones were tested for tolerance to heat in the summer season in Israel (Levy et. Al., 1991). Fifteen plants of each cultivar or clone were grown in pots, in sterilized potting mixture in a 50 mesh screenhouse to avoid aphids and damage by insects. The results are presented in Table 12.

Nievsky proved to contain tolerance to heat, Nievsky clone 5 being the most tolerant.

5.13. Virus studies in Israel

During this project plantlets and minitubers originating from Astana wete tested in Israel for presence of viruses. Thus, in 1998 ten plantlets var. Nievsky grown from meristems (in tubes), were sent to Israel and tested by ELISA for PVX, PVY, PVS and PVM and were found free from these viruses. The plantlets were also tested for PVX on *Gomphrena globosa* and found to be negative. This indicates that the method for obtaining virus free plantlets by meristems in Akmola seems reliable.

Also in 1998, minitubers from Nievsky sent from Akmola were sprouted under quarantine conditions in Israel and tested by ELISA for the above-mentioned viruses. They were also tested on *G. globosa, Nicotiana glutinosa, Chenopodium amaranticolor* and *Cucumis sativus*. All 12 minitubers that sprouted did not give any symptoms on the test plants and gave negative reactions by ELISA. Three plants were given to Dr. Levy's labs for rapid propagation.

Testing of Akmola plantlets with the Dig-labeled RNA probe to *Potato leaf roll virus* (PLRV) showed that all the Akmola plantlets tested with this probe were negative.

Further studies were done to check the method (Loebenstein et al. 1997, Plant Disease 81: 489-491): to reduce the size of tissues samples. No. marked differences were observed when the samples were reduced from 50 µl to 3 µl. The Dig-labeled RNA probe for PLRV was further evaluated in various samples of potato tubers and was found to detect the virus reliably.

Formamide was evaluated as a denaturing agent instead of formaldehyde but was not found satisfactory. It was regularly observed that the hybridization buffer has to be freshly prepared before use.

During this period additional antisera for PVX, PVY, PVS and PVM were prepared. Some antisera were sent to Kazakhstan.

6. Impact Relevance and Technology Transfer

As a result of this cooperation the laboratory in Astana succeeded to improve its propagation technology and especially its virus testing procedures. They also changed there basic approach that meristeming in itself is not sufficient to obtain virus-free plants, but that it is necessary first to obtain virus-free mother plants, which will then serve for the rapid propagation. They also got more expertise in the ELISA testing, and realized that students cannot carry out such tests, but these have to be done by qualified technicians. It also became evident that antisera produced in Almaty were more sensitive

than those obtained from Moscow. Nevertheless, in spite of the very difficult situation in Astana their achievements were remarkable and high quality seed tubers were obtained. The resulting increase in potato yields led to a major interest both from the agricultural experts of the Kazakh Ministry of agriculture and Kolkhoz farmers.
The laboratory in Almaty gained much experience in purification of potato viruses and preparation of antisera. Their antisera is of high specificity with almost no background reactions and a very high titer. The purification techniques that they adapted have been distributed to several other laboratories in Kazakhstan and are employed there successfully. They had enquiries from
DOE-Pacific Northwest Laboratories (PNNL), Richland,WA, USA.-to produce for them antisera for potato viruses..
There was also an interest from a Kazakh firm to go into producing seed potato as well as from a Kyrgyz farming company. However, due to the investment needed and the high interest, their interest did not materialize. Attempts to obtain loans from international funding agencies did not realize, as they do not handle loans or grants in the range of 1-3 million $.
In addition to seed production, Neivsky's field performance and tuber quality for processing was evaluated.
Nievsky (clone 5) was shown to be tolerant to heat and of average yielding capacity in Mediterranean climate. Due to its round shape, it can be used for chips production although the chips could have some browning because of some sugars in the tubers. This is very desirable in view of the growing demand for chips in many countries.

7. Project Activities/Outputs

During this period Ms. Ishenko Oksana received her diploma from the Agricultural Faculty of Astana. Her thesis was " Methods of obtaining virus-free potatoes" under guidance of Dr. V. Shvidchenko.Mr. Andrey Buenkov worked as a post-graduate student in Dr. Shvidchenko's lab on methods of rapid propagation.
Dr. Alija Manadilova from Almaty spent a training period in Dr. Levy's lab from March 8, 1999 till August 8, 1999 studying : 1. Rapid propagation of potatoes; and 2. Transformation of peppers. (See attached reports 3 and 4).
A paper entitled "Rapid propagation of virus-tested potatoes in Kazakhstan" was presented at the 14th International Plant Protection Congress, Jerusalem, July 1999 (Abstract 5 attached).

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8. Project Productivity

The project achieved its goals. A system of rapid propagation of virus-tested Niewski potatoes was established in Astana. Within this part of the project a substantial amount of Elite tuber seeds (about 660 tons) were produced. These "elite" seed gave an increase in yields of about 90% compared to commercial fields in the vicinity, i.e. 25.3 t/ha compared with 13.3 t/ha, respectively. However, as Dr. Shvidchenlo left Kazakhstan it is questionable if this project will continue now in Kazakhstan, but may probably continue in Krasnodar, Russia.
The expertise gained in the Institute of Molecular Biology and Biochemistry in Almaty in purification of potato viruses, preparation of high quality antisera and developing a sensitive ribiprobe for virus detection will continue to be an asset for Kazakhstan.

9. Future Work

Eventually this project will be continued, i.e. the production of virus-tested seed potatoes by rapid propagation and the production of quality antisera. The first objective will best be continued through a joint venture arrangement between a commercial company and a research institute. It is also expected that Dr. Shvidchenko who moved to Krasnodar in Russia, will develop a similar project there. Production of antisera may continue if a need for them arises. Upscaling of the system requires the involvement of the Kazakh government. An official laboratory for testing and diagnosis of viruses and other pests, commercial laboratories for rapid propagation, facilities for large scale 'elite' tuber production, specific areas for seed multiplication in the field by specialist growers all are now needed to facilitate local seed production in Kazahkstan.

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Attachments

1. Potato virus M. (PVM).

Potato virus M was purified in from tomatoes(Tomato talalichina), inoculated with a field isolate, checked with PVM antiserum and free from PVX, PVY. PVS and PLRV. Leaves were extracted about one month after inoculation in 0.2M hosphate buffer, pH 7.6, containing 0.2% thioglycolic acid, 3% urea and 1% Triton X-100. After low speed centrifugation the supernatant was extracted with an equal volume of Chloroform: Tetracarbon and after separation, the water phase was ultracentrifuged on a 20% sucrose cushion containing 3% urea. The pellet was resuspended in 3 ml 0.1M phosphate buffer pH 7.0, subjected to low speed centrifugation and centrifuged through a 10-40% sucrose gradient. The virus containing fractions were ultracentrifuged and the pellet resuspended in 1 ml of 0.1M phosphate buffer, pH 7. From 125 g of infected tomato leaves between 0.76-1 mg of PVM were obtained. The preparation was checked by PAGE and found to be without detectable plant protein. These preparation were injected into rabbits and an antiserum was obtained that had a titer of 1:2000 for both coating and conjugate.
The yield of PVM obtained was 4,6 mg frpm 100 gr.leaves.

2.Antiserum production.

Antisera were produced by injecting rabbits at 3 week intervals with approx. 1 mg purified virus mixed with an equal volume of Freund 's incomplete adjuvant . Rabbits were immunized by a combination of subcutaneous and<intramuscular injections and were bled at 1 week intervals after 2 to 4 injections. Antisera were stored at -18 C, either frozen or mixed with 50% glycerol. 1:1000 titters were determined.
The conjugate of immunoglobulins with an alkaline phosphatase (Sigma) was prepared by a method : IgG (1 ml) was mixed with alkaline phosphatase (225 mkg (2,500 IU)) and dialyzed against potassium phosphate buffer at 4°C during night, after that to the mixture was added glutaraldehyde (to final concentration 0.05 %) and incubated 4 h at room temperature then dialyzed against potassium phosphate buffer at 4°C during night. The obtained conjugates stored at 4°C with adding 1 % sodium aside.
The sandwich-ELISA carried out according to Clark and Adams. Absorbence at 405 nm was measured using an ELISA-reader (BioTek, EC – 310).

3. Experiments on pepper transformation

(Report by Dr. A. Manadilova for her study period in Israel March 8-Auguast 8, 1999)
The effect of different treatments on transformation efficiency were studied.
Experiments were conducted with several cultivars: Maor, California Wonder, Galron and a Hungarian cultivar.
In all cases accept in Experiments 3 & 6 mature embryos were used. Agrobactem/m-mediated transformation efficiency was followed with the transient expression of the GUS reporter gene.
Experiment 1: The influence of plasmolysis.
Maltose in the medium, at concentrations rage of 1 .OM-0.09M, was used as osmoticum.
Range of duration of osmotic treatments was 1,2. or 3h.
Application time of plasmolysis treatments was: 1) Pretreatment before infection of embryos with *Agrobacterium.* 2) Plasmolysis and infection with *Agrobacterium* starting at the same time. 3) Plasmolysis starting as a pretreatment before infection and continuing during first hours of infection with the bacterium. Experiments on points (a)-(c) were conducted on pepper Maor.
Effect of plasmolysis by 0.6M maltose was studied on all pepper types. Experiment 2: The influence of prolin. or betaine or prolin & betaine given together.

Betaine was given at 1mM, Prolin at 1mM]. Prolin was applied at 3% or 0.6M maltose as osmotic treatment.
Experiment 3: The influence ultrasonication.
The ultrasonication times were 1 ot 10 sec. The experiment was conducted on detached cotyledons of 14 days old seedlings.
Experiment 4: The influence of plasmolysis, 25mM prolin and zeatin at 0.3, 3.0 or 30 mg/L.
The test was conducted with embryos of Galron and C.W. * Experiment 5: The influence of growth temperature of *Agrobacterium.*
Agrobacterium was incubated for different times (3,7,10 or14 days) in 4°C or 25°C. The embryos were infected and cocultured foer 5d in the growth room.
Experiment 6: Agrobacterium-mediated trasformation by flower dip.
Omametal pepper X was grown in the greenhouse to flowering. At flowering plants were dipped in an *Agrobacterium* suspension including either 50 ml/L or 500 ml/L Silvet 77. Plants were dipped 3 times: Once when all first flower buds were dosed, and the second and third dip at intervals of one week.

4.Rapid propagation of potatoes.
(Report of Dr A. Manadilova for her study tour in Israel).
One of the most important factors in the rapid propagation is the quality and vigor of the transplants which evolve from the cuttings of the in vitro plantlets.
Tubers were planted in pots in the greenhouse. Each plant were tested for viruses Those who found virus free were the source for tissue culture, the others were discarded . Meristem culture of potatoes was practiced to facilitate the use of this technique for the establishment of virus-free nuclear stocks. In the first step about 100 cuttings were obtained from the virus tested" base" plants. The stem shoots, after removal of the leaves, were sterilized (1 min in 70% ethanol, followed by 20 min in 20% Ca OC1 and then rinsed with sterilized water,,and placed in tubes or small plastic containers containing agar plus Murashige and Skoog medium with the addition of sucrose. All the work were done aseptically in laminar air-flow cabinets. It is expected that within 5 months about 40000-50000 plantlets were obtained. These plantlets will be used for producing plantlets which can be directly transplanted to the field and for growing minitubers.
The following was done during period from 8 March to 8 August.
1. The plants of potatoes(Kazahstan, Israel and USA species) free from viral infection were propagated by subculturing 3 times in MS - medium.
2. Techniques for excision of meristems from shoot tips was learned
3. Techniques for measuring alkaloids in potato tubers were learned. The content of alkaloids and the dry weight in different genotypes of potato were compared.

5. RAPID PROPAGATION OF VIRUS-TESTED POTATOES IN KAZAKHSTAN – Abstract of presentation at the International Congress of Plant Protection, Jerusalem 1999.
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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.

Through meristem cultures and heat therapy virus-tested plants of the varieties Nievsky and Tamasha were prepared. These plants were propagated by tissue cultunng and "minitubers" were prepared in Astana. In 1998 about 50,000 miniyubers were planted in Northern Kazakhstan yielding 28 tons/ha , compared with 14 tons/ha in commercial fields in this region.
This scheme of rapid propagation and "minitubers" shortens markedly the number of generations from the "nuclear" plants to "certified seed" and he exposure to re-infection by viruses.
This work was supported by a US AID grant within the CDR/CAR program.

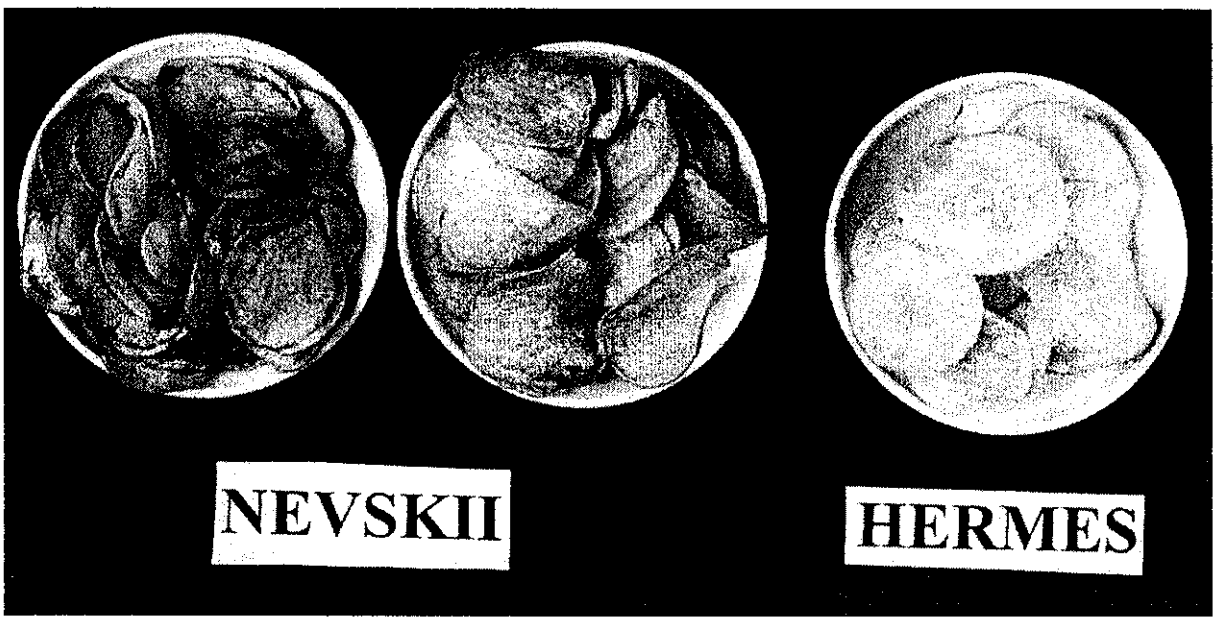

Figure 1. Chips of the clones 3 and 5 of Nvievsky.

Table 11. Field performance of Nievsky (clones 3 and 5) as compared to control cultivars.

Cultivar	Haulm vigour	Resistance to fros frost	Tuber yield (kg/m2)	Dry matter in tubers (%)	Sugar content in tubers (%)	Tuber shape	Tuber skin Color	Flesh Color	Growth Cracks
Nievsky (3)	good	good	1.8	17.3	0.25	round-oval	yellow	yellow	some
Nievsky (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

Table 12. Performance of potato clones and cultivars grown in the summer season in Israel. Idit, Ori, Zohar, Zahov, 6120, 6087, 1000, E5-145, BARD1-3 and BC631 were selected in Israel for tolerance to high temperatures. N565.1, A85560-8, Nievsky 3 and Nievsky 5 were introduced as clones. Nicola and Mondial served as control cultivars.
S. tuberosum = tbr; S. andigena = adg; S. phureja = phu; S. demissum = dms; S. acaule = acl; S. chacoense = chc; S. microdontum = mcd; S. vernei = vrn; S. berthaultii = brt; 2x = diploid.

Cultivar or Clone	Species (Including ancestors)[a]	Origin	Haulm weight at harvest (g/plant ± SE)	Tuberization (%)	Tubers/plant ± SE	Tubers yield (g/plant ± SE)	Diameter range of large tubers (mm)
Idit	tbr	Israel	0.85 ± 0.11	7	1	10	-
Ori	tbr	Israel	1.47 ± 0.21	27	5.1 ± 1.2	61± 24	25-45
Zohar	tbr	Israel	2.35 ± 0.15	27	3.8 ± 2.4	44 ± 13	20-45
N565.1[b]	adg	U.S.A	2.03 ± 0.29	62	1.6 ± 0.3	18 ± 15	15-30
A85560-8[c]	tbr, acl, adg, dms	U.S.A	1.72 ± 0.22	66	8.3 ± 2.0	160 ± 42	30-50
1000[d]	tbr, adg, phu, ber	U.S.A – Israel	2.25 ± 0.17	93	6.4 ± 1.0	139 ± 27	30-60
Zahov[d]	tbr, adg, phu, chc	U.S.A – Israel	1.68 ± 0.11	100	10.8 ± 1.1	197 ± 33	25-55
6087[d]	tbr, adg, mcd, phu, chc	U.S.A - Israel	2.01 ± 0.11	27	2.8 ± 1.1	12 ± 6	20-25
E5-145[e]	tbr,adg,dms	U.S.A – Israel	2.20 ± 0.22	13	3.5 ± 1.5	40 ± 10	30-40
Nievsky-3	tbr,adg,dms	Kazakhstan	0.84 ± 0.10	80	6.7 ± 0.7	117 ± 19	20-50
Nievsky-5	tbr, adg, dms	Kazakhstan	1.13 ± 0.08	93	9.2 ± 0.8	252 ± 37	30-60
Nicola	tbr, dms	Germany	1.31 ± 0.18	57	5.6 ± 1.1	60 ± 27	20-30
Mondial	tbr, dms, vrn	Netherlands	2.22 ± 0.14	0	0	0	-
6120[f]	tbr (2x)	Israel	1.66 ± 0.14	64	4.8 + 1.0	74 ± 27	15-45
Bard 1-3	phu (2x)	U.S.A – Israel	1.02 ± 0.41	11	24	37	15
BC631	phu, chc (2x)	U.S.A	1.78 ± 0.32	64	17.3 + 3.8	276	18
C80-1	chc (2x)	U.S.A	2.23 ± 0.07	25	1	0.3	5

[a]According to Stegemann and Schnick, 1985; Plaisted and Hoopes, 1989; personal communications.
[b]Introduced from the International Potato Center, Lima, Peru
[c]Introduced from the University of Idaho, Moscow (R.B. Dwelle)
[d]Selected in Israel from true potato seed received from R.E. Veilleux
[e]Selected in Israel from true potato seed received from R.L. Plaisted, Cornell University, N.Y.
[f]Dihaploid (2x) from Desiree