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TITLE OF PROJECT

Characterization of viroids infecting fruit trees in Kazakhstan

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Executive summary

Research focused on two main subjects with relevance to the project aims:

(I) Development of a novel Northern hybridization probe (Multiprobe) aimed to detect several different viroids

(II) Attempts to identify possible natural means of viroid dispersal to fruit trees and vines

The common finding of two or more viroid infections in many fruit trees and the need to eliminate all these viroids, suggested that the use of a new type of probes capable of simultaneous detection of several different viroids would facilitate practical diagnosis of viroid and other virus diseases of fruit trees. Our work focused on the construction of hybridization vectors capable of detection of *Hop stunt viroid* (HSVd) and *Citrus exocortis viroid* (CEVd), two economically important viroids widely distributed among different fruit trees.

The common finding of multiple viroid infections among the majority of vegetatively propagated fruit-trees and vines from this region was considered mainly to result of horizontal transmission within propagation materials and/or mechanical transmission on grafting and pruning tools. However, none of the horticultural means of spread, could had explained the presence of the hop stunt viroid (HSVd) among most of the native and exotic fruit trees and grapevines from this region. Our work demonstrated for the first time the ability of goats to transmit fruit tree viroids from infected to healthy plants. These findings are expected too have a serious impact on future interactions between horticulturists and grazing animals in Kazakhstan and throughout many other developing countries

SECTION I

A) Research Objectives:

(I) Development of a novel methods for viroid detection and their use for surveys and fruit tree certification and disease elimination in Kazakstan

(II) Develop means to identify and to prevent natural and other possible sources of viroid spread among fruit trees in Kazakstan and other developing countries

B) Research accomplishments: Work in the first part of this year focused on the use of real time PCR for viroid detection. Primers for sensitive detection were designed and tested for several viroids. Later studies showed that under routine research conditions where PCR is used for a variety of laboratory needs such as diagnosis, cloning and sequencing many samples might turn to result in misdiagnosis as false positives. This prompted us to change the direction of detection from PCR to Northern hybridization. Initial assays showed that the method is highly sensible, can be performed with partially purified extraction samples from a variety of plant tissues. As a follow up of these results we shifted our efforts now to widen the range of viroids, which will be detected by a single multipurpose probe

Our work focused on the construction of hybridization probes capable of simultaneous detection of both *Hop stunt viroid* (HSVd) and *Citrus exocortis viroid* (CEVd), two economically important viroids widely distributed among different fruit trees.

The common finding of multiple viroid infections among the majority of vegetatively propagated fruit-trees and vines from this region was considered mainly to result of horizontal transmission within propagation materials and/or mechanical transmission on grafting and pruning tools. However, none of the horticultural means of spread, could had explained the presence of the hop stunt viroid (HSVd) among most of the native and exotic fruit trees and grapevines from this region. Our work demonstrated for the first time the ability of goats to transmit fruit tree viroids from infected to healthy plants. These findings are expected too have a serious impact on future interactions between

horticulturists and grazing animals in Kazakhstan and throughout many other developing countries

Development of a novel Northern hybridization probe (Multiprobe) aimed to detect several different viroids

The common finding of two or more viroid infections in many fruit trees and the need to eliminate each of these viroids, suggested that the use of probe capable of simultaneous detection of several different viroids would be of considerable use for practical diagnosis. At the present our work focuses on the construction of hybridization vectors capable of detection of Hop stunt and Citrus exocortis viroids, two economically important viroids widely distributed among different fruit trees. Various parameters including the size of the hybridization signal and the need of spacers are under investigation. It is expected that this project will be sufficiently furthered within the next 4-5 months to allow the demonstration of the new probes to our Kazak cooperators during the visit and viroid detection course planned with Dr. A. Hadidi to take place during June 2004.

Attempts to locate the possible natural means of viroid dispersal to fruit trees and vines

Our recent experiments demonstrated the ability of goats to acquire and transmit viroids to herbaceous indicator plants by scratching their horns first on stems of infected fruit trees. Hybridization assays showed that viroids could persist for several days on contaminated horns. The ability of goats to vector viroids provides a plausible explanation to the wide range distribution of several different viroids among different fruit trees and vines

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explained the presence of the hop stunt viroid (HSVd) among most of the native and exotic fruit trees and grapevines from this region. The recent finding of genetic identity between viroid from wild pear trees from remote hills in Greece and those from domesticated cultivars suggested that in this region there should also be an alternative route of natural infection.

The possibility that goats are able to transmit two viroids *citrus exocortis viroid* (CEVd) and *Hope stunt viroid* (HSVd) was tested by feeding a group of female Saanen goats (n=8) on two large (3 yr) Rangpur lime plants, infected with the #225 isolate of CEVd). Feeding was continued until the acquisition plants were defoliated and their bark had been severely scraped. Feeding was then continued on a group (n=8) of one-year sour orange (SO) healthy seedlings. After dressing the severed SO bark with parafilm, plants were incubated in a temperature-controlled cabinet with temp of 29 and 23 C during day and night time, respectively. Infection was tested by chip-grafting tissue of the SO plants on the Ethrog indicator plants and RT-PCR analysis for the presence of CEVd. None of the indicator plants showed symptoms, and PCR analyses did not reveal CEVd in the grafted indicators (not shown).

Since viroids could persist for long periods on mechanical tools like razor blades and grafting knives, we tested the possibility that goat-horns could had also served as natural platforms for viroid transmission. Northern hybridization analyses of RNA extracts from shavings of goat horns that were scratched 24 hrs earlier with Ethrog citron stems infected with the # 225 complex gave intensive signals for both HSVd and CEVd. Signal intensity of RNA extracts from different Goats differed several fold and could be apparently correlated with the apparent differences in surface structure of the goat horns. Following these experiments that suggested that goats might be involved in natural transmission of fruit viroids, these experiments were extended now to study the possibility of HSVd and CEVd transmission to healthy fruit trees. The implication of these results on viroid transmission and elimination in Kazakhstan and other countries were extensive goat and fruit tree cultivation takes place will be discussed with our Kazak partners.

C) Scientific Impact of Collaboration: As indicated above our research focused on developing the tools for detection of viroids and proving the vector potential of goats.

The diagnostic tools will be transferred as a package to our Kazak partners and will find immediate use there for viroid detection and elimination

D) Description of Project Impact: The impact on Kazak horticulture will only be felt after technology transfer will take place in June.

E) Strengthening of Developing Countries Institutions: This aspect of our project will filled with the training on viroid management and control and transfer of detection tools that will take place during June 2004 with the visit of Dr. Hadidi and Dr. Bar-Joseph in Kazkstan

F) **Future work:** 1 Development of diagnostic multiprobe capable of detection of all known fruit tree viroids. 2. Use of multiprobes for viroid detection in native and cultivated fruit trees from Kazakstan.

SECTION II

1. **Managerial Issues**: Mr. Rosa Gofman, a former technician in this laboratory, specializing on viroid extraction left the team and was replaced by Mr. Roni Cohen who is conducting now his Master thesis on viroid detection in fruit trees.
2. **Budget**: The economy of budgeting the Israeli partners was helped by the fact that expenses on Student thesis work is far lower then technical assistance.
3. **Special Concerns**: All our detection work is conducted with non-radioactive work, animal experiments (Viroid transmission on goat horns) are conducted under natural conditions and do not involve any intervention with the normal life of goats.
4. **Collaboration**: We are continuously interacting by mail with Dr. Hadidi and wrote jointly a short paper on viroids in the Middle East, published recently in a Book on viroid Edited by Dr. Hadidi et al, 2003. We expect collaboration

with our Kazak partners to improve considerably following the visit and training that will take place in June 2004

5. **Request for American Embassy Tel Aviv or AID actions**: (I) The project included the training of a Kazak researcher in viroid detection techniques, visa entrance problems for a six months training was not obtained yet, despite the invitation letter sent to Kazakhstan and a result no such training was provided. (II) A visit of training and lectures on basic and practical aspects of viroid detection and elimination is planned during June 2004. Dr. A. Hadidi a former USDA employee and one of the world leading experts on fruit tree viroids and originally a consultant on this project expressed his readiness to participate in this training trip. I would like therefore that the cost of his trip and per diem in the range of 5000 should be paid from the 8500 $ travel money allocated for the Israeli partners in this project. A formal letter will be sent to Mr. Boaz Ayalon, the coordinator of this project