Discussion. There was no found full identity of primary structure among each four Russian PSTVd and any other earlier known PSTVd. All four Russian isolates have one joint distinction from Intermediate isolate: deletion adenine in position 123 (variable region). Three isolates originated from Leningradskaya province (North-western region) were closely related: All of them had substitution of adenine in position 120 (conservative region), but in the case of isolate Onega-Premier-94 and Niva-95 adenine was replaced by uracyl and in the case of isolate Bugry-97 by cytosine.

Isolate Samara 97-3 was received from the Volga-river region that is far distant from Leningradskaya province. In comparison with Intermediate isolate the isolate Samara 97-3 had the same changes in variable and conservative region of RNA molecule as isolate Bugry-97, but in addition to those, it had 2 changes in variable region. Adenine in position 310 was replaced by uracyl, and additional uracil was inserted in position 313a.

When changes affected conservative and variable regions, a character of host plants response to infection remained the same, that is, intermediate-type. When changing took place in pathogenic region (310 A→T, 313a T insert), disease induced mild symptoms. Changes in pathogenic region produced more stable double-helix structure (Figure 3).

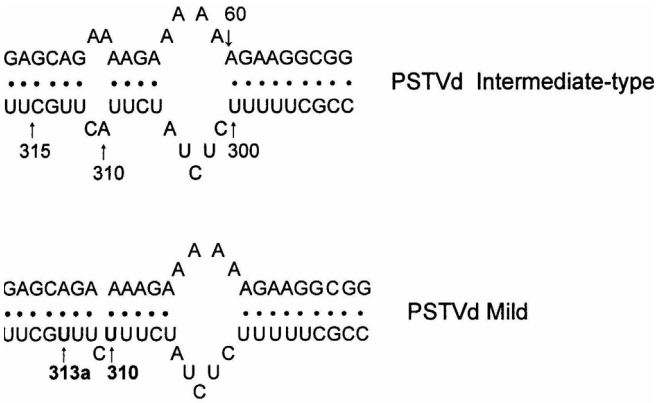

Fig. 3. Fragments of PSTVd sequence of pathogenic region (44-68, 292-317) for intermediate-type and mild strains of PSTVd (see *B. Lewin. Genes VI. Oxford – N.Y. – Tokyo, Oxford University Press, 1997. P. 145*).

When we say about severe, intermediate or mild symptoms, usually the matter concerns symptoms on tomatoes as indicator plants (cv. Rutgers), because symptoms on potato plants may differ. If PSTVd infected potato has been maintaining for many years, accumulated infection of mild PSTVd strains may induce intermediate or severe symptoms.

Additional studies with a wider range of Russian isolates of PSTVd are currently underway.

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PLANT VIRUSES AS BIORESOURCES IN TURKEY AND ESTABLISHMENT OF TURKISH PLANT VIRUS COLECTION

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Turkey is known as motherland for many plants. Research/study show that number of plant viruses and virus-like agents are present in Turkey and some of them firstly have been founded in turkey such as CCDV. There is need to conserve virus and virus-like pathogen as bioresources in a suitable way and facilitate of making further study for developing continual and successful control strategy against plant viruses. An in-situ collection started to be constructed from 2006 with collecting genetic resources of virus and virus-like agents. For this aim a general survey and biological, serological, histological and molecular detection activity is performing and then the founded agent will be conserved in green and screen houses at PPRI in Adana. At the moment, more than 10 virus and virus-like agents have been founded, detected and placed into collection.

Introduction. Virus and virus-like pathogens are world wide distributed and generally cause severe loses and economical damage during plant production. The control of these diseases is based on identification and diagnosis of the causal agents. Many research activities have been taken for this reason and many virus and virus-like diseases have been reported from Turkey. There is need to conserve this type of pathogen in a suitable way and make further study for developing continual and successful control strategy.

As well known is a crucial point to have genetic recourse of the viruses for the scientific work and this source has importance from strategically point of view. There is a big need to have a virus and virus like diseases collection for Turkey because of Anatolia is not only one of the most important gene sources for plant spices but also for the plant pathogens.

Since five decade years many scientific study has been done on plant viruses and many plant viruses have been reported from different locations on different hosts in different time. Some of those reported viruses are presented at table.1.

Table 1. **The list of some plant virus diseases reported from Turkey mainly on woody hosts.**

Name of virus	Genus	Acronym	Author & date
Citrus tristeza	*Closterovirus*	CTV	Baloğlu, 1988.
Grapevine leaf roll-associated	*Closteroviruses*	GLRaV1,2,3,7	Köklü 1999.
Citrus psorosis virus	*?*	CPsV	Güllü, 1987.
Citrus chlorotic dwarf	*?*	CCDV	Korkmaz, 1994.
Christacortis	*?*	CCV	Yılmaz, 1998.
Amasya cherry diseases	*Partitivirus*	ACD	Covelli *et.* all, 2004.
Apple chlorotic leaf spot	*Trichovirus*	ACLSV	Cağlayan, 2003.
Grapevine A	*Trichovirus*	GVA	Ciğsar *et* al. 2002.
Prune dwarf	*Ilarvirus*	PDV	Cağlayan, 2003.
Prunus necrotic ring spot	*Ilarvirus*	PNRSV	Cağlayan, 2003.
Apple stem grooving	*Capillovirus*	ASGV	Birisik *at* all., 2006.
Apple stem pitting	*Foveavirus*	ASPV	Birisik *at* all., 2006.
Pepper Mild Mottle Virus	*Tobamovirus:*	PMMV	Güldür et al., 1994.
Tomato mosaic	*Tobamovirus*	ToMV	Yılmaz and Davis, 1984.
Cucumber Vein Yellowing Virus	*Ipomovirus*	CVYV	Yılmaz, 1989.
Cucumber mosaic	*Cucumovirus*	CMV	Yılmaz , 1976.
Lettuce big-vein	*Varicosavirus*	LBVV	Erkan *at* all., 1985.
Tobacco etch potyvirus	*Potyvirus*	TEV	Yılmaz *et* al. 1983.
Potato virus Y	*Potyvirus*	PVY	Ekbiç *et.,* al.,1997.
Plum pox	*Potyvirus*	PPV	Cağlayan, 2003.
Bean common mosaic	*Potyvirus*	BCMV	Güzel ve Arlı-Sökmen, 2003.
Bean common mosaic necrosis	*Potyvirus*	BCMNV	Güzel ve Arlı-Sökmen, 2003.
Watermelon mosaic 1	*Potyvirus,*	WMV-1	Yılmaz and Davis, 1985.
Watermelon mosaic 2	*Potyvirus*	WMV-2	Erdiller and Ertunç, 1988.
Zucchini yellow mosaic	*Potyvirus*	ZYMV	Yılmaz and Davis, 1985.
Eggplant mottled dwarf	*Nucleorhabdovirus*	EMDV	Palloix, *et al.,* 1994.
Cucurbit Aphid Borne Yellow Virus	*Polerovirus*	CABYV	Fidan, 1993.
Alfalfa mosaic alfamovirus	*Alfamovirus*	AMV	Fidan, 1993.
Squash mosaic	*Comovirus*	SqMV	Yılmaz and Davis, 1985.
Cauliflower mosaic	*Caulimovirus*	CaMV	Erkan *et al.,*1991.
Lettuce big-vein	*Varicosavirus*	LBVV	Döken *et al*., 1993.
Grapevine fleck virus	*Maculovirus*	GFkV	Köklü, 1998.
Tobacco ringspot	*Nepovirus,*	TRSV	Fidan, 1993.
Tomato black ring	*Nepovirus*	TBRV	Fidan, 1993.
Arabis mosaic	*Nepovirus*	ArMV	Akbaş and Erdiller, 1993
Grapevine fan leaf	*Nepovirus*	GFLV	Akbaş and Erdiller, 1993.
Strawberry latent ring spot	*Nepovirus*	SLRSV	Akbaş and Erdiller, 1993.
Grapevine Anatolian ring spot virus	*Nepovirus*	GARSV	Gökalp et al, 2003.
Cherry leaf roll	*Nepovirus*	CLRV	Çağlayan *et al*., 2004.
Grapevine virus	*Nepovirus*	GNV	Köklü, 1998.

Materials and methods. Plant virus isolates which are collected during the survey activity and some other isolates which have been accepted from other research institute and universities. Herbaceous and woody indicator plants have been used for mechanical inoculation, woody indexing and for grafting. ELISA reagent and ELISA reader used for serological detection of viruses. RNA extraction kit (Promega SV Total RNA Isolation System), PCR thermocyler, gel visualizing system for molecular assays. Screen house and green houses which are present in APPRI, for producing plant material, and to keep infected material in-situ. Climatic rooms have been used for plant production activity and maintain some virus isolates which can be spread out to nature via insect vector or some way else. Sterile growing media for indicators, Carborandum dust, grafting knife, different type of label, grafting wax, mortar, liquid nitrogen, air-conditioner, cheese clothes, nicotine, different germination media and hormones. Some insecticides, disinfectants, fertilizer, ice etc.

I. Random surveys

Random surveys were carried out during the all field visits in case of research activity and also during the pest control surveys. Samples have been taken by visual observation of the suspected plants.

II. Targeted specific surveys

Location targeted surveys have been performed according to the plants and pathogens up-to their best time for symptom expression and good time for testing. Collected samples have been taken form different part of suspected plants (Leaf, shoots, flowers, fruits) and transferred to the laboratory in appropriate conditions (into a plastic back in the ice box) for using in testing methods. Meanwhile some plant cutting included buds have been collected and conserved for grafting.

III. Provide plant seeds and indicator plants

Indicators plants and their seeds have been supplied form different research Institutes and universities such as Yalova Atatürk horticulture research institute and University of Cukurova.

IV. Breeding of seed and indicator plants

Mature plant seeds maintained in torf as one layer seed and one layer torf at 0-4.4 $C^{o,}$ for 120-130 days and then they sowed in 100x50x25x cm size boxes. When seed germinated they have transplanted to plastic bags or pots which include sterile growing media include sand, torf and soil in (1:1:1) mixture rate. Herbaceous plants have been produced in climatic rooms in conditions of 8/16 hour Light/dark period under 3000 lux supplemented light and free from any insect and mite infection.

V. Samples taking from inoculum sources

The samples taken form different site and different part (leaf, shots, bark and fruits) of suspected trees and samples will be transferred in plastic bags at 4 C^{o} to laboratory. Samples will be maintained at 4 C^{o} and then tested as soon as possible by serological and molecular means. Cuttings which have been used for biological assays, grafting for inoculation and for rooting have be wrap up with humid serviette paper and keep at the 4 C^{o} until use.

VI. DAS-ELISA

DAS-ELİSA is performed by using different commercial (Agritest, Bioreba, Loewe) antisera kits. ELISA tests done mainly according literature [4] with some differences advised by antisera producer companies. Absorbance is read after 15 min, 30 min, 60 min, and 90 min, using automatic plate reader at 405 nm absorbance (Titertek Multiskan plus MKİİ reader).

VII. Mechanical Inoculation

Sap transmissions activity has carried out using 8 herbaceous hosts belonging to 5 different families. Young or mature leaves from infeted plants, are come from the survey, are used for sap inoculation to different host plants as shown in Table 2. The leaves are extracted in 0.05 M phosphate buffer (pH 7.0). The herbaceous plants are kept at 24-27°C and daily inspected for specific symptom [20].

Table 2. **Herbaceous test plants are used in mechanical inoculation**

Nicotiana accidenatlis
Nicotiana tabacum
Phaseolus vulgaris
Vigna unguiculata
Cucumis sativus
Chenopodium amaranticolor
C. quinoa
C. album

VIII. Biyological Indexing

Indexing can be defined as any test that reproducibly assess the presence and absence of a transmissible pathogen or identify a disease on the basis the reactions induced on specific indicator plant. Woody indicator plants some other plants suitable for a certain virus diseases have been inoculated by patch and bud grafting [8].

Table 3. **List of woody indicator are used for indexing**

Mexican lime
Sweet orange
Sour orange
Prunus persiaca cv. GF305
Malus sylvestris cv. R12740-7A
Malus sylvestris cv. Virginia Crab
SPY 227
Lord Lambourne

IX. RNA extraction from the plant tissue and PCR assays

Total RNA extraction was performed by using commercial isolation kits. Isolation was done according to manufacturer advising guide (Promega SV Total RNA Isolation system) and [17]. Mainly plant phloem and leaf tissue was used. Isolated tRNAs were used in RT (Reverse transcriptase) assays in presence of M-MLV reverse transcriptase. Produced cDNA was used in PCR for amplification. PCR reactions was prepared according to each viruses based on the scientific literature. Primers were designed by IONTEK ltd. şti. All other components of the PCR such as Taq-polymerase, dNTPs, buffers were used from commercial (all from Promega).

PCR products were visualized by electrophoresis in 1.5 % agarose gel stained with 0.5 ug/ml of ethidum bromide. Basic RNA isolation and cDNA production was done according [17].

X. Maintain and observation of the infected plants in greenhouse and screen house

The viruses which are subjected to the quarantine and has potential to spread out form the collection such as CTV and PPV have been placed in the climatic rooms where have no any direct open and locked usually. Viruses have potential to spread by any natural means such pollen like PNRSV have been placed in greenhouse. If there is no any natural transmission possibility for a virus such as ASGV and ASPV they have been placed in screen house.

Rooted cutting used for some plants are suspected to be infected with any viruses such olive and fig which have no any special plant indicator for virus testing. 3-5 infected cuttings (about 30 cm long) have been taken and dipped into 3000 ppm IBA (Indol butyric aside or Naphthalene acetic aside) solution to incite rooting. Rooted cuttings transformed to plastic bags (25 x 15 cm) for adaptation to the soil.

All plants either grafted or naturally infected, they have been weekly observed for the presence of the symptoms expression after they placed in the in-situ collection.

During the weekly control, all of the plants also have been controlled for the presence of any pest and diseases. In any doubt of pest infection required pesticides have been sprayed.

Results and discussion. After two years survey more than thousand plants were visually observed and 231 suspected plants were sampled. 196 samples were tested by ELISA for several viruses of fruit trees. RT-PCR assay were proceed for 19 samples out of 231 which gave suspected result in ELISA or had no any result in ELISA even they have some symptoms indicated that virus infection. During two years surveys 7 different viruses have been founded, tested and placed in the screen house or green house according their etiology. Those are Citrus tristeza *closteravirus* (CTV), Prunus necrotic ring spot *ilarvirus* (PNRSV), Apple chlorotic leaf spot *trichovirus* (ACLSV), Apple mosaic *ilarvirus* (ApMV), Apple stem grooving *capillovirus* (ASGV), Apple stem pitting *foveavirus* (ASPV) and Fig mosaic virus disease.

Apart form the samples have been taken during activity, 29 samples have been accepted via phtytoclinc laboratory to test for any virus infection. Form those plants Prune dwarf *Ilarvirus* (PDV) have found in a local prune variety. This PDV isolate have been grafted on virus free GF-305 indicator and placed in screen house. Citrus psorosis virus (CPsV) also has been found in an infected grapefruit sapling came to institute by the grower. This CPsV isolate have been tested by ELSA and then grafted on sweet orange and both infected plants placed in screen house.

Plum pox potyvirus PPV infected buds were provided from University of Cukurova and grafted on four individual GF 305 plants and placed in screen hose under the isolation box.

In the APPRI in-situ collection 10 plant viruses are present as grafted on woody plant neither source plant nor indicator. The viruses and number of infected plant is shown at table 4. To well-care of plant and to keep them form any other infection is a very hard, but to have live infected plants is useful goods for performing detection tests and observation virus symptoms.

Table 4. **Virus diseases are present in the APPRI in-situ collection**

Virus	Hosts	Indicator	Tested by,	Number of plant
CTV	Citrus spp.	Mexican lime	1,2,3	2
CPsV	Citrus spp	Sweet orange, grapefruit	1,2	4
PNRSV	Prunus spp Rosa sp.	*Prunus persiaca* cv. GF305	2,3	5
PDV	Prunus spp	*P. persiaca* cv. GF305	1,2	2
ACLSV	Malus spp. Prunus spp.	*Malus sylvestris* cv. R12740-7A	1,2	4
PPV	Prunus spp	*P. persiaca* cv. GF305	1,2,3	4
ASGV	Malus spp.	*Malus sylvestris* cv. Virginia Crab	2,3	8
ASPV	Malus spp. Pyrus spp.	SPY 227	1,2	8
ApMV	Malus spp.	Lord Lambourne	1,2,3	3
FigMV	Ficus spp	Ficus carica	1	3

1: Detected by visual observation
2: Tested by ELISA
3: Confirmed by RT-PCR

Construction of an in-situ collection means a killing pathogen is keeping in a plant which is diseased and it may die. It needs to spend a big effort to for production of virus free indicators/hosts, for continuously re-inoculation and to care about the inoculated plant. Isolation of the virus free material and infected plants from the internal and external pathogens is very vital. Detection of the single infection in a certain individual plan is very hard for some spices which have tendency to be infected with more than one virus like grape.

If a quarantine viruses such as CTV and PPV are detected is very difficult and risky to manage and to keep it in a safe place for avoiding any diffusion. Some viruses are very latent like ASGV so that it is very hard about to be sure that artificial inoculation to the indicators is succeeded.

Viruses are pathogens and also they are biorisources from the nature. To have enough number of viruses and enough isolates of a certain viruses will give a great opportunity to work and to use them in developing detection methods, testing for cross protection and use in molecular biology.

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VIRUS DISEASES OF VEGETABLE CROPS IN GREENHOUSE CONDITIONS IN UKRAINE

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Spreading of viruses of vegetable group in greenhouse conditions in Ukraine were analyzed. Serological and electronic microscopy data identified 3 viruses infecting vegetable crops: Cucumber green mottle mosaic virus (CGMMV), Impatiens necrotic spot virus (INSV) and Turnip mosaic virus (TuMV). From these, two viruses: INSV and TuMV have been detected in Ukraine for the first time.

Introduction. Vegetable-growing in greenhouses is one of the most intensive forms of agriculture that gives the possibility to obtain significant yield from square unit almost all year round. Hence specific greenhouse conditions: absence of crop rotation, unchangeable utilization of soils, absence of the species and hybrids with group resistance to diseases, artificially created microclimate in greenhouse and other factors cause favorable conditions for mass development of different bacterial, fungal and nematode diseases [2]. But the most serious danger for the greenhouse is provided by viruses, as they cause about 50% of yield loss, and in some cases the yield is lost completely [3]. For today about 18 species of viruses of different taxonomic affiliations are registered on the cultures of cucumbers, peppers and tomatoes in greenhouse conditions. These viruses are transmitted by seeds, insects, soil solution and throught the contact of leaves. Lately, new observations indicate definite widening of areals for major virus infections, spread of complex and latent diseases, appearance of their novel forms with altered pathogenicity [4]. Studying of species diversity of viruses and ways of their propagation will give the possibility to limit the area of their spreading and as a result to increase the yield productivity of crops.

Thus, our research was focused mainly on the analysis of modern situation of spreading of viruses that infect vegetable grops in greenhouses conditions in Ukraine.

Object and methods of investigation. The object of the study were the cucumbers, peppers, egg-plants and tomatoes with typical viral symptoms that have been obtained from the different greenhouses of Ukraine.

Sample analysis for presence of virus antigens has been carried out employing sandwich and indirect ELISA modifications. ELISA has been carried out in polystyrene plates "Labsystem". Results were registered using 'Dynatech' reader at the wavelength 405 nm [5].

Isolation of viruses from the infected plants has been carried out by homogenization of symptomatic plant leaves in 0.1M PBS + 0.001M EDTA in proportion 1:2 (w/v), and filtra-