Azerbaijan National Academy of Sciences
Institute of Botany

SEROLOGYCAL AND MOLECULAR DETECTION OF MELON (*Cucumis melo* L.) INFECTING VIRUSES IN AZERBAIJAN

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Introduction

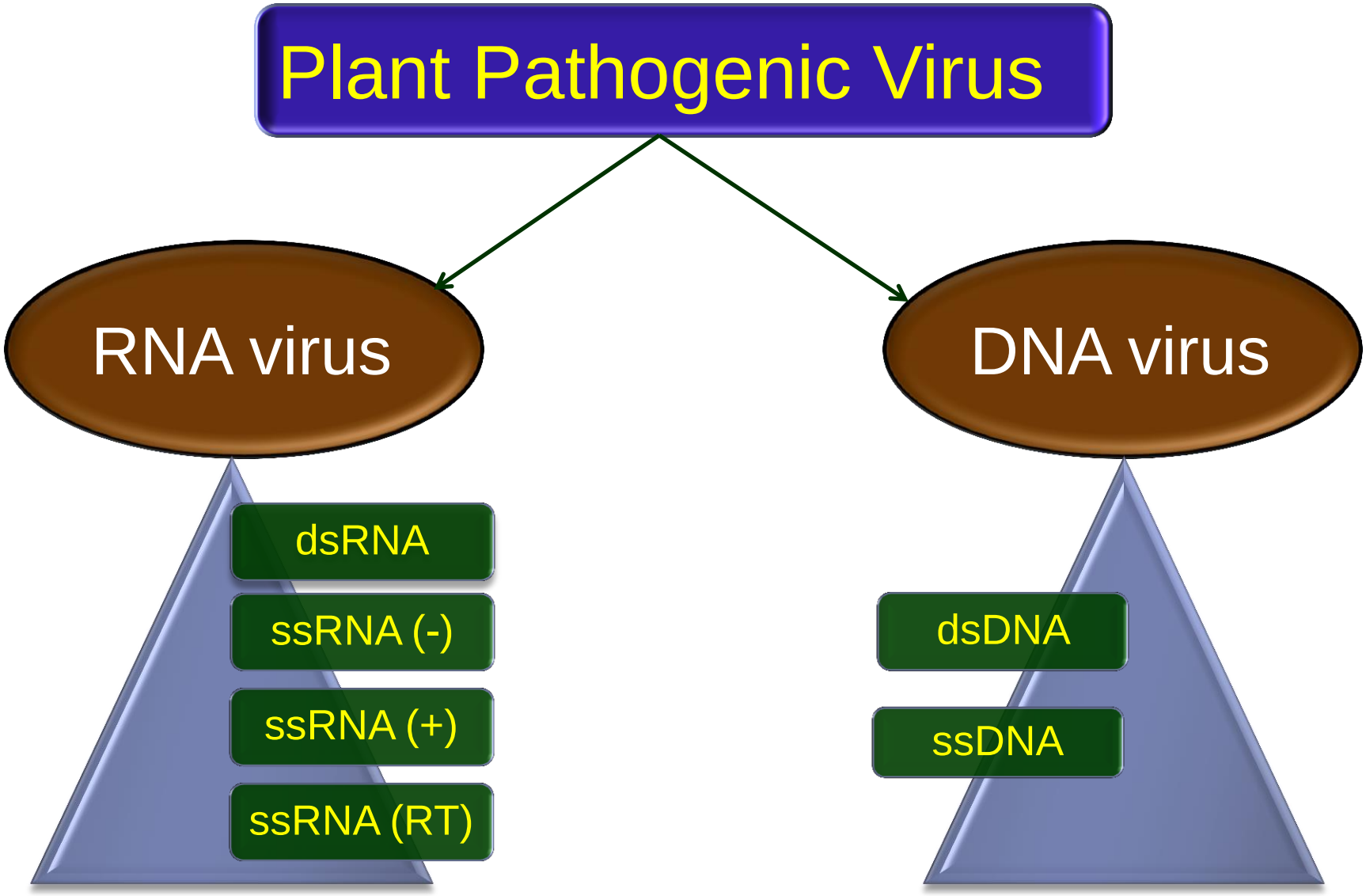
- **Virus diseases are one of the major constraints for higher production of crop plants.**
- **Heavy losses in yield of crop plants due to viruses especially in vegetables.**
- **Combined infestation of more than two viruses at time in a particular crop.**
- **The incidence and severity of different virus diseases on different crops are in increasing trend.**
- **Economic yield losses in various crops are not known.**
- **Sometimes 90% yield loss was observed in Tomato crop.**
- **Farmers experiences more than 90% yield losses in cucumber, other cucurbits and tomato crops**
- **Virus disease management particularly in vegetables and leguminous crops are most essential.**



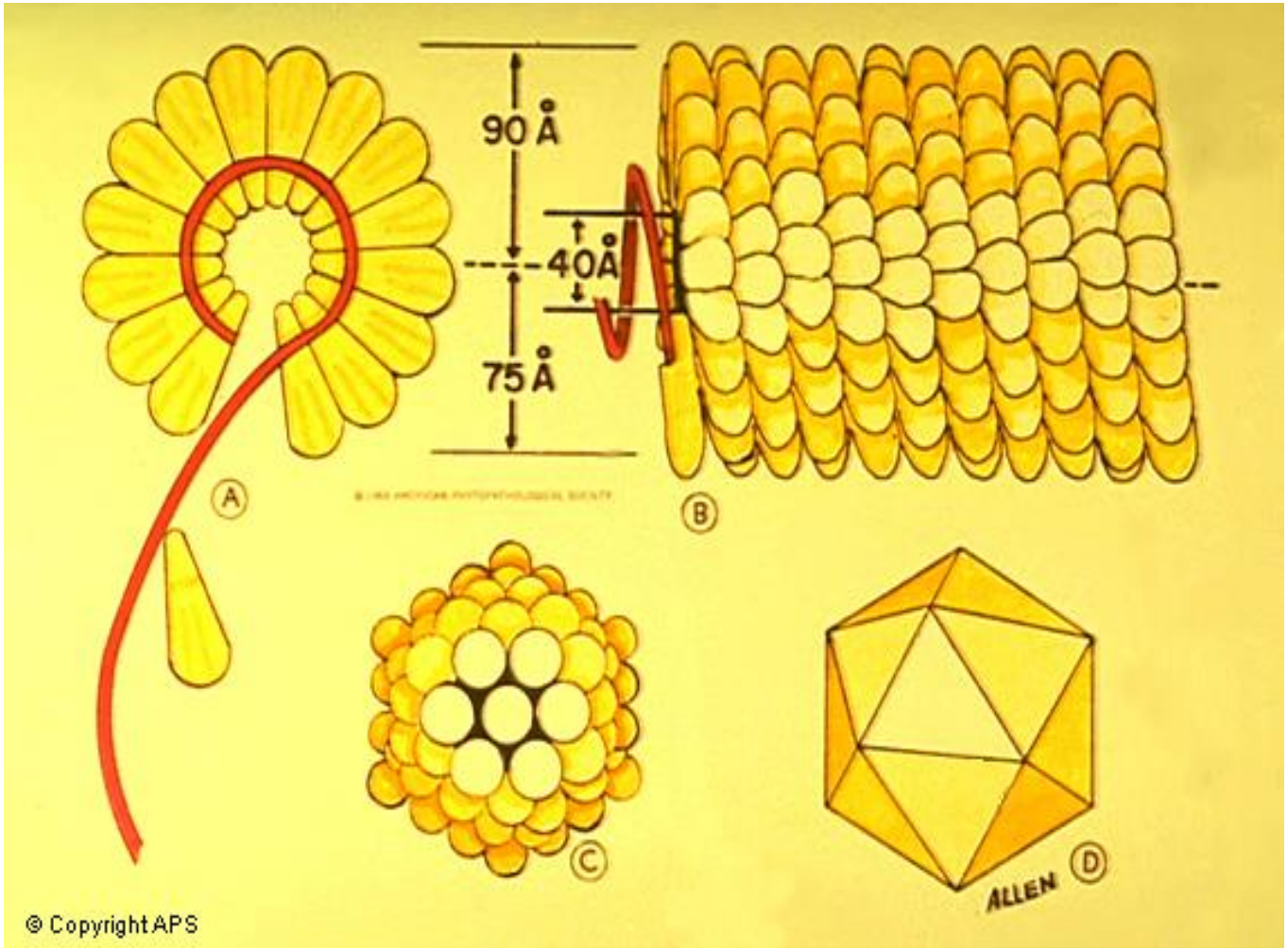
Plant virus genome organizations

- Very compact
- Most are + sense RNA viruses, so translation regulation very important
- Use various strategies for genome expression
- Only a few genes absolutely required:
  - Replicase
  - Coat protein
  - Cell-to-cell movement protein
- Other genes present in some viruses




Plant Pathogenic Virus
RNA virus
DNA virus
dsRNA
ssRNA (-)
ssRNA (+)
ssRNA (RT)
dsDNA
ssDNA

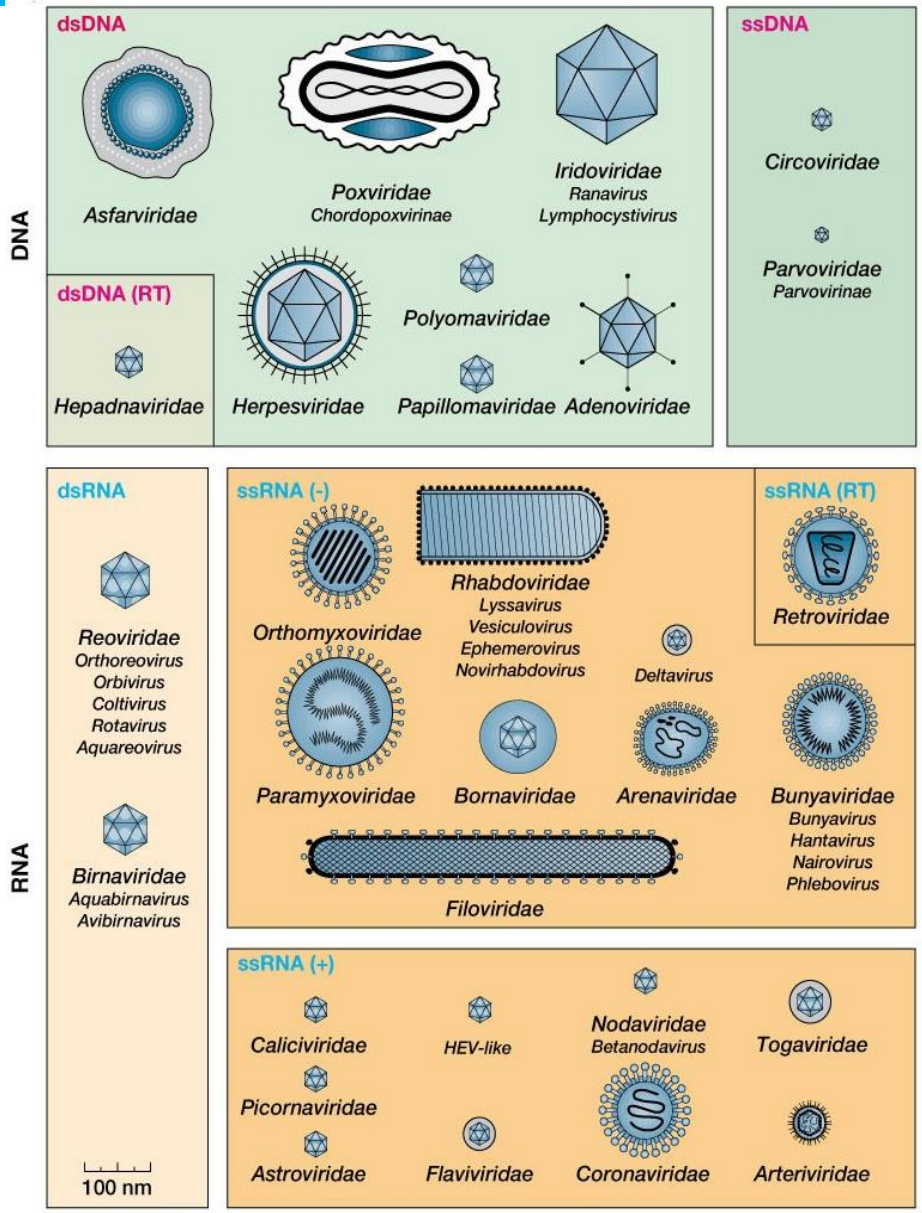
Basic Plant Virus Structures

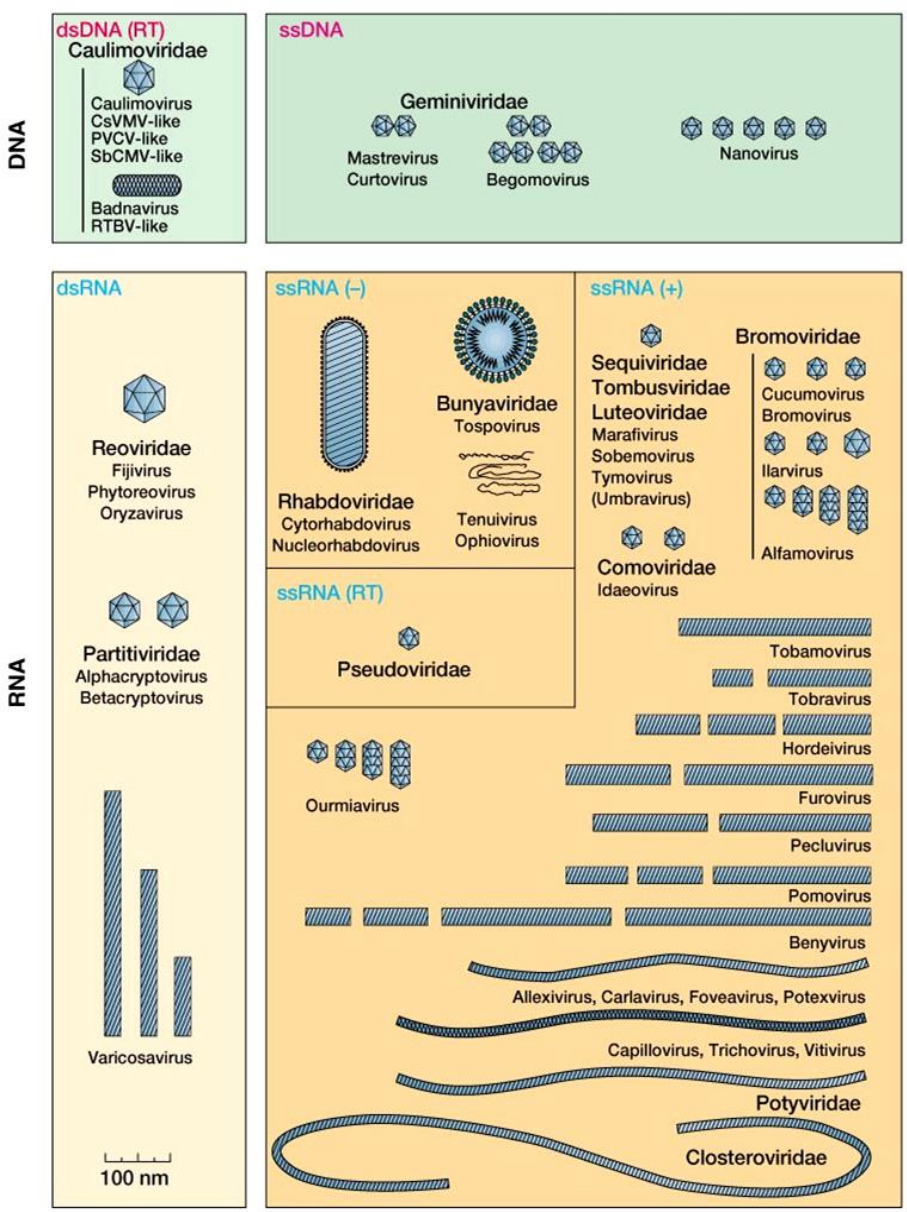


Helix (rod)
e.g., **TMV**

Icosahedron
(sphere)
e.g., **BMV**

Comparisons of Animal and Plant Viruses





Animal Infecting Viruses

Plant Infecting Viruses

Cucurbit, tomato and pepper viruses

✓>70 species, 20 viral genera infect cucurbits

✓>100 species(*), 32 viral genera infect tomato (11 major viruses)

✓>60 species(*), 32 genera infect pepper (12 major viruses)

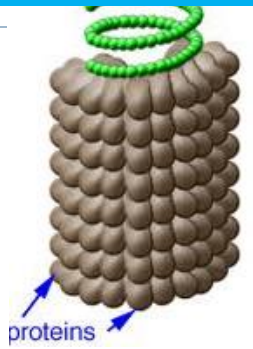
(*) not including all begomoviruses (>50 species, taxonomy unclear)

40 viruses in common between tomato and pepper, very few between cucurbits and solanaceae

✓In the Europe countries: in the last 35 years, one « new » virus reported on the average every year



Plant viruses

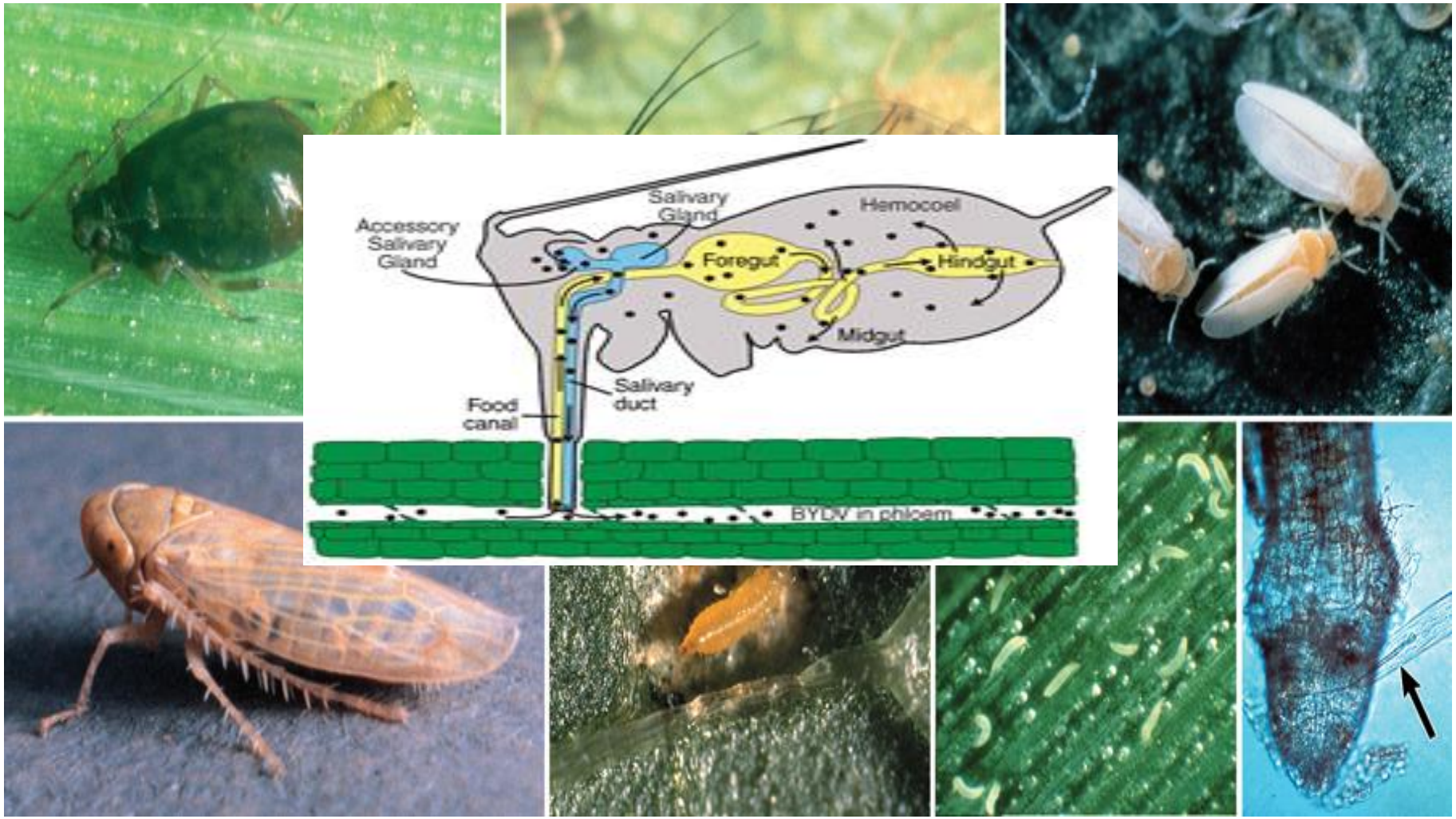


- ✓Genome: RNA in 80% of plant viruses
- ✓Small genome (3 to 20 kb)
- ✓No conserved region in all known viruses
- ✓High mutation rate
- ✓Transmission usually by vectors (mostly insects)

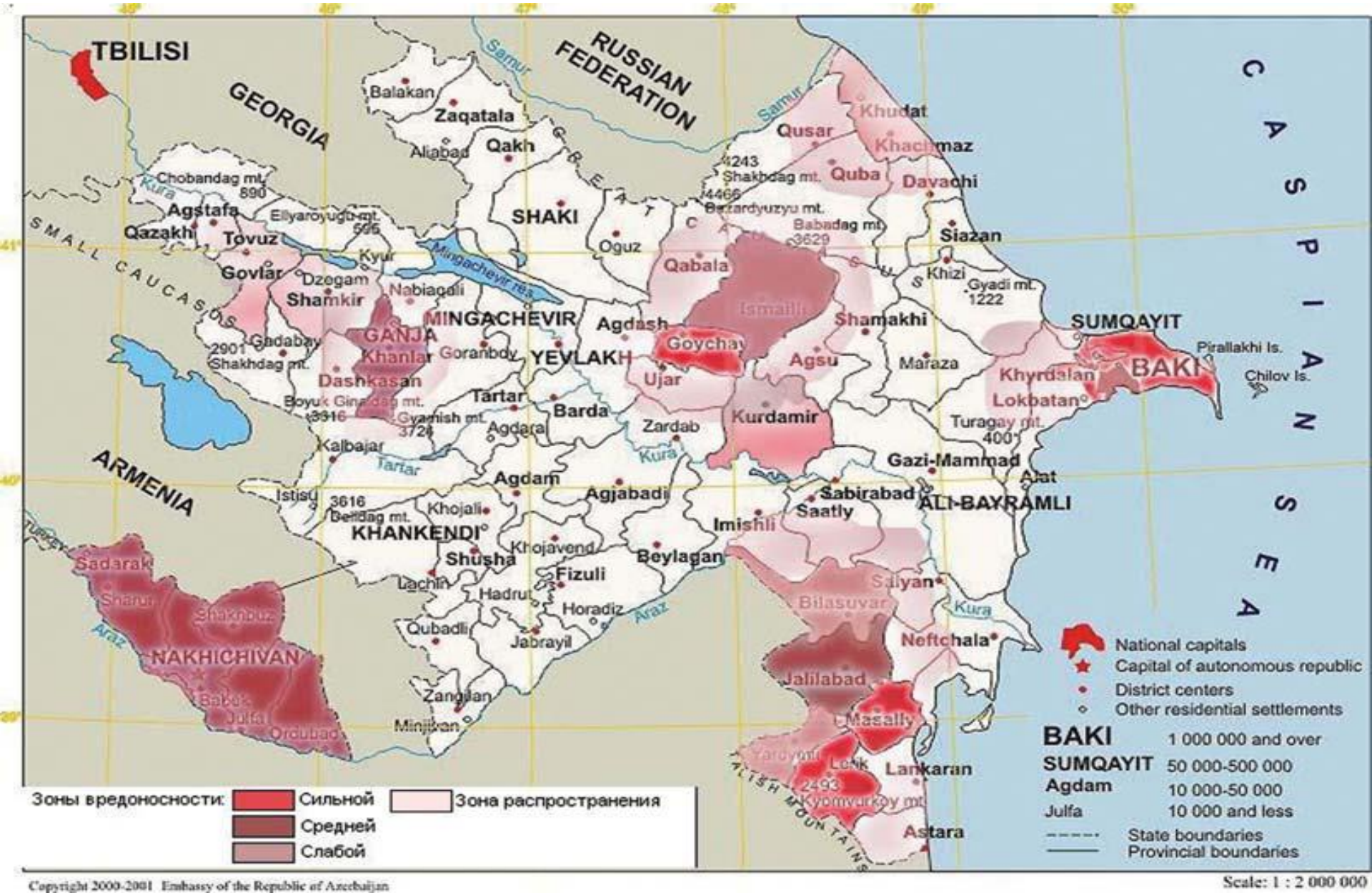





Vectors of plant viruses: **A**-wingless aphid. **B-** winged aphid. **C**- whiteflies. **D-** leafhopper. **E-** thrips (larva). **F-** eriophyid mites. **G-** migratory ectoparasitic nematode



Field trip on the main crop production regions of Azerbaijan



Description of Azerbaijan Field Situation

Symptoms observed in the field:

High incidence of plants with virus-like symptoms

Randomly distributed symptoms in the field

- **Stunted growth as a common symptom, especially when mix of viruses are present**
- **Leaf distortion, bubbly, yellowing, mottling and mosaic patterns**
- **Young leaves curl downwards, brown streaked leaves**



Plant Virus Symptoms

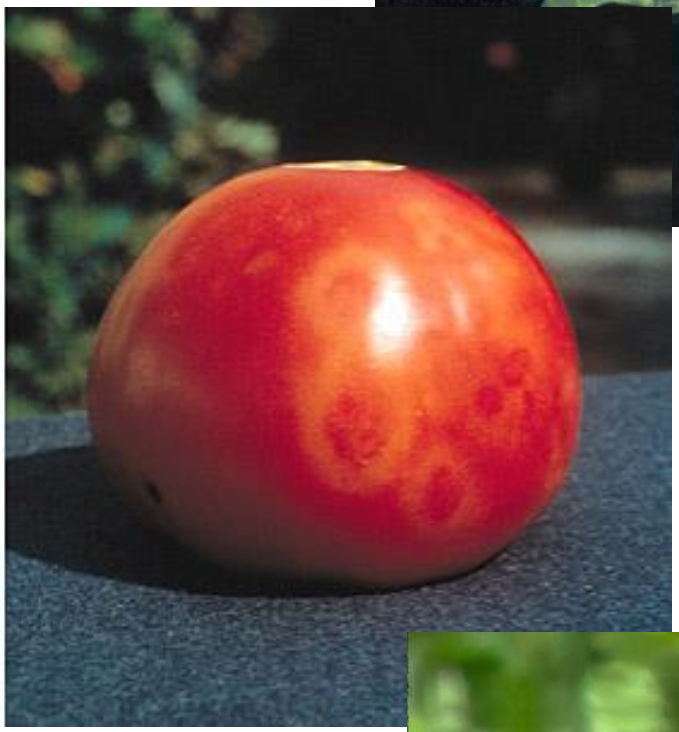
- Stunting - common
- Mosaics & mottles - common
- Ringspots - common
- Abnormal growth/tumors/enations - rare
- Blights - rare
- Wilts – rare
- Systemic necrotic lesions – relatively rare



Mosaics – very common







Tomato mosaic



Alfalfa mosaic



Necrotic lesions

Leaf mosaic and leaf roll



Tomato bushy stunt virus (*TBSV*)







Leaf yellowing





Destroyed and reduced fruit quality







Leaf yellowing, growth reduction







Shortening the life-span, extensive leaf coloring and stunting



Leaf yellowing and leaf roll





Leaf distortion

- Mixed infections



Cucumber mosaic virus, CMV

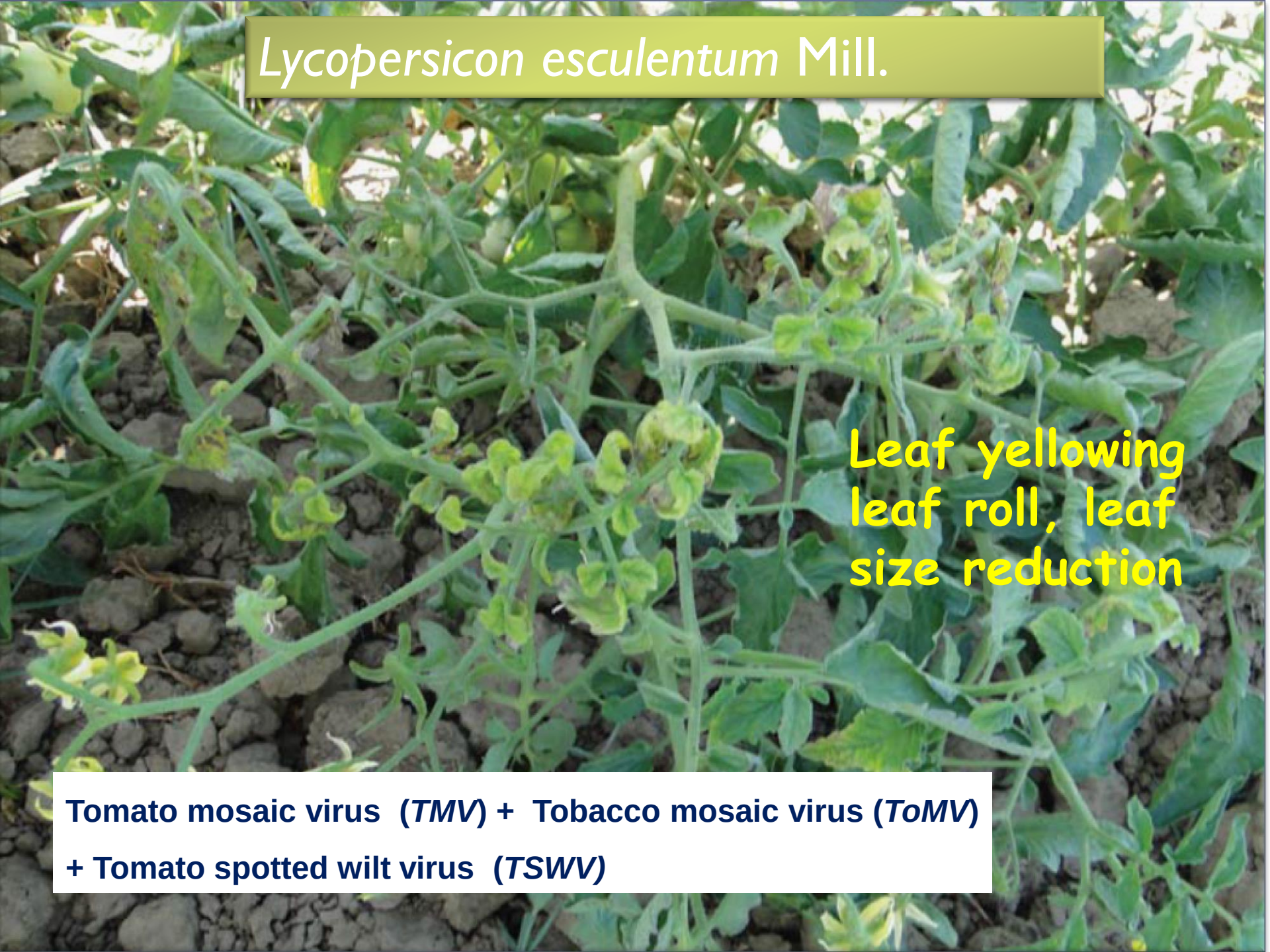


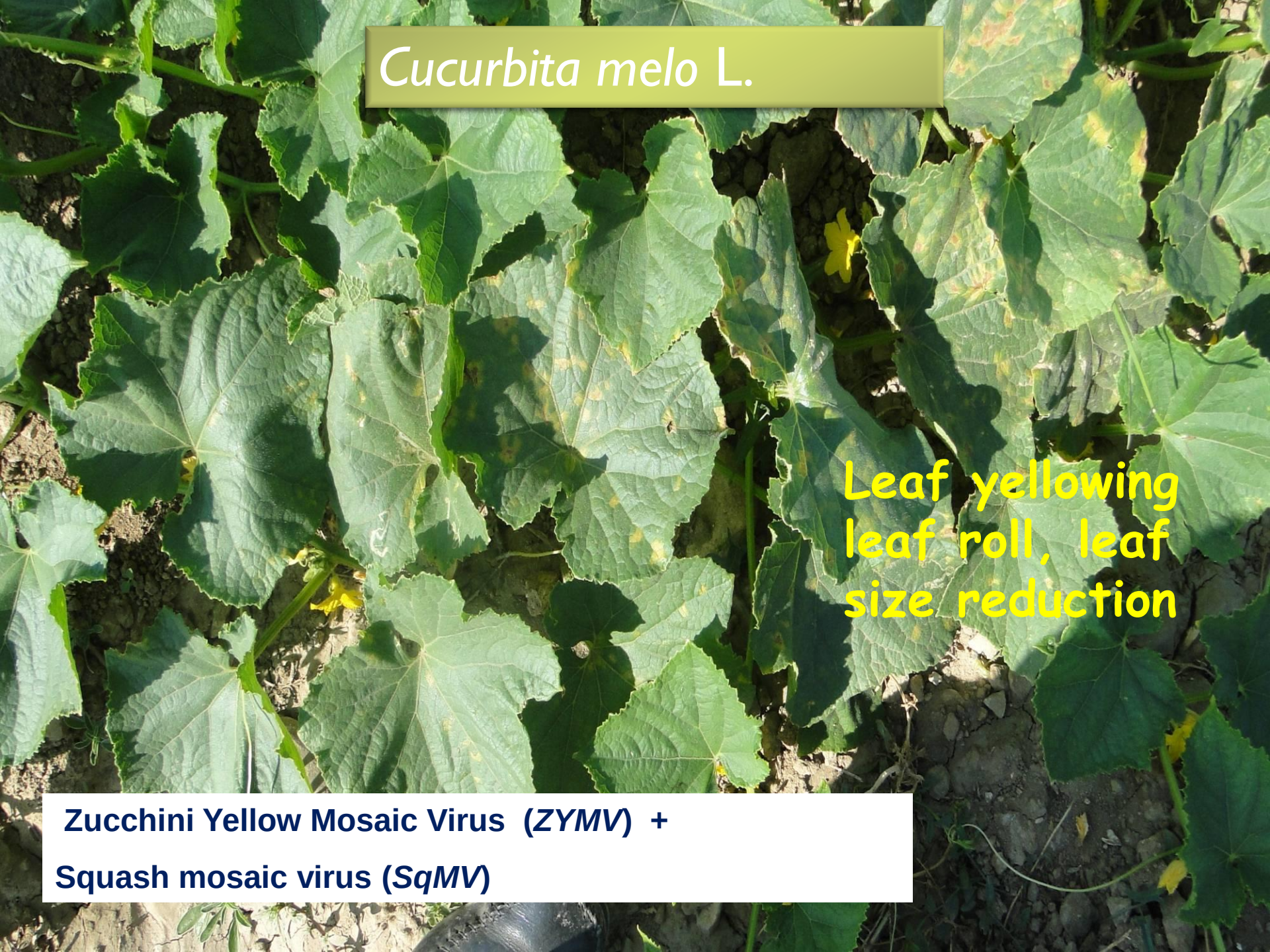
Watermelon mosaic virus, WMV

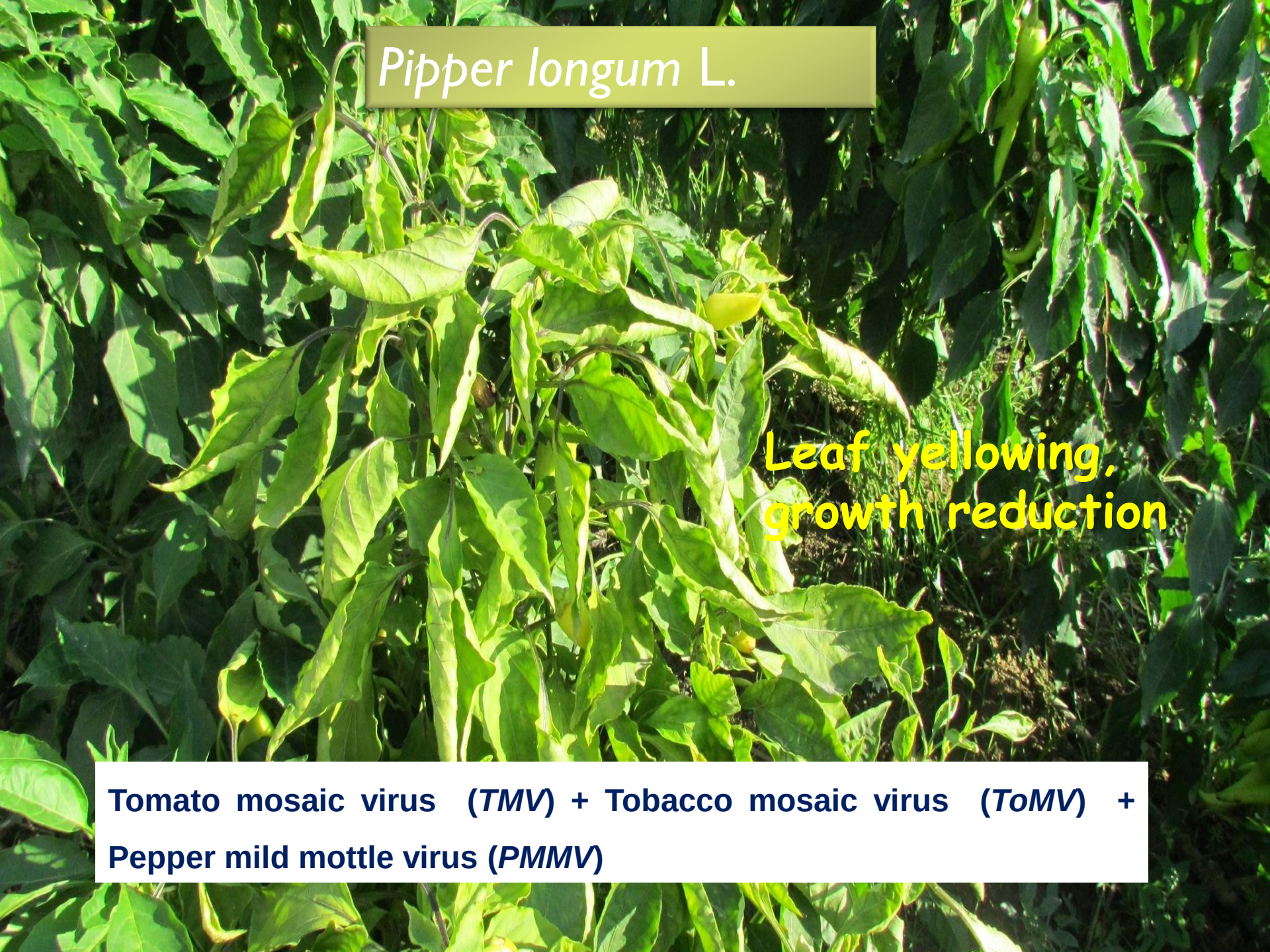


CMV + WMV

Photos : © H. Lecoq INRA


Lycopersicon esculentum Mill.
Leaf yellowing leaf roll, leaf size reduction
Tomato mosaic virus (*TMV*) + Tobacco mosaic virus (*ToMV*)
+ Tomato spotted wilt virus (*TSWV*)


Cucurbita melo L.
Leaf yellowing leaf roll, leaf size reduction
Zucchini Yellow Mosaic Virus (ZYMV) +
Squash mosaic virus (SqMV)


Pipper longum L.
Leaf yellowing, growth reduction
Tomato mosaic virus (TMV) + Tobacco mosaic virus (ToMV) + Pepper mild mottle virus (PMMV)

Most common « classic » viruses infecting cucurbits



- ✓ *Cucumber mosaic virus* (CMV, *Cucumovirus*)



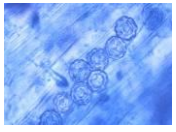
- ✓ *Watermelon mosaic virus* (WMV, *Potyvirus*)



- ✓ *Zucchini yellow mosaic virus* (ZYMV, *Potyvirus*)



- ✓ *Cucurbit aphid borne yellows virus* (CABYV, *Polerovirus*)



- ✓ *Melon necrotic spot virus* (MNSV, *Carmovirus*)











Emerging viruses (whitefly-transmitted)



- ✓ *Criniviruses: Cucurbit yellow stunting disorder virus* (CYSDV), *cucumber chlorotic yellows virus* (CCYV)





- ✓ *Cucumber vein yellowing virus* (CVYV, *Ipomovirus*)





- ✓ *Begomoviruses: Watermelon chlorotic stunt virus (WmCSV), Squash leaf curl virus (SqLCV), Tomato leaf curl New Delhi/Palampur virus (ToLCNDV, ToLCPaV)…*



What is the situation in Azerbaijan?

Previous surveys:

- In 2003 on cucurbits (30 samples from Landekan and Khaçmac regions): ZYMV, CABYV, WMV, CMV, MNSV

- On tomato: 2009-2010: possible presence of TYLCV, not confirmed (Grigoras et al., 2010)

In neighbouring countries (Iran):

- Cucurbits: aphid-transmitted viruses (ZYMV, WMV, CABYV, CMV…) + whitefly-transmitted (begomoviruses: TLCPaV, ToLCNDV, WmCSV; CVYV, CYSDV, CCYV) + others (OuMV, CLSV, CGMMV…)
- Tomato: aphid-transmitted, nematode-borne, thrips-borne, mechanically transmitted viruses (CMV, TMV, ToMV, TAV, PVY, TMGMV, ToRSV, TSWV) + whitefly-transmitted (TYLCV + other begomoviruses)



Surveys: Sept 12-19, 2014

September: end of growing season (maximum of virus infections)







Surveys: Sept 12-19, 2014

September: end of growing season (maximum of virus infections)

Many symptoms observed on cucurbits, tomatoes and pepper (few on eggplant)

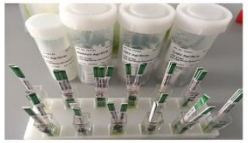







Diagnostic of Azerbaijan samples

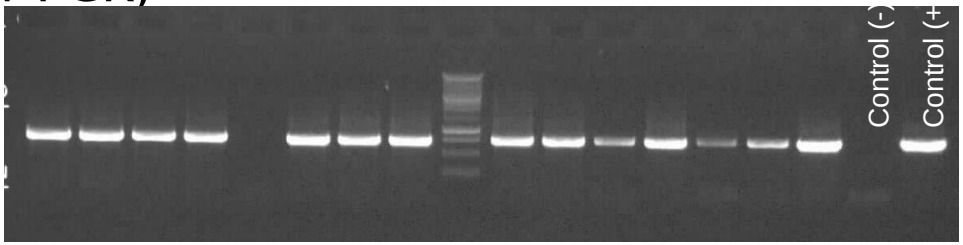





- Sample collection based on symptom observations
- Serological tests (DAS-ELISA):
  - Cucurbits: 16 antisera
  - Tomato & pepper: 8 antisera




- When no antiserum is available: molecular tests (RNA or DNA extraction, RT-PCR or PCR)

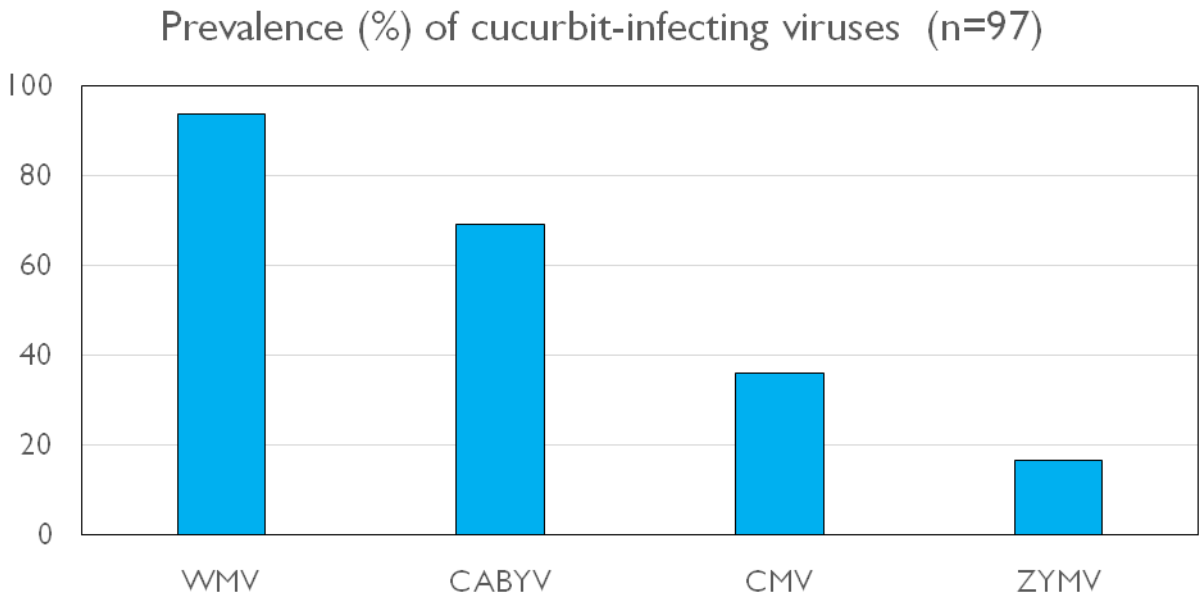


- Mechanical inoculations
- Electron microscopy

Screening for cucurbit viruses

Viruses tested by DAS-ELISA: ZYMV, WMV, PRSV, MWMV, CMV, CABYV, CVYV, CYSDV, BPYV, MNSV, SqMV, OuMV, EMDV, SqLCV, WmCSV, CGMMV (16 species, 10 genera)

➔ 4 viruses detected, high prevalence of WMV



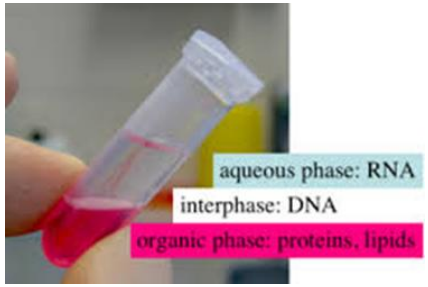
Virus tested by RT-PCR (no antiserum available for ELISA): CCYV (present in Iran)

➔ Not detected

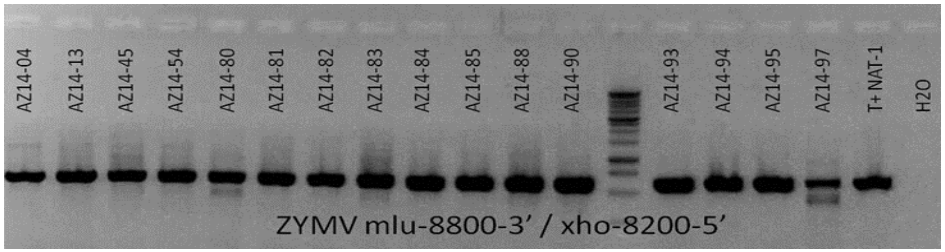
Are AZ isolates closely related to those of neighbouring countries (common origin?) or have they evolved independenlty?

➔Molecular characterization of cucurbit viruses from Azerbaijan

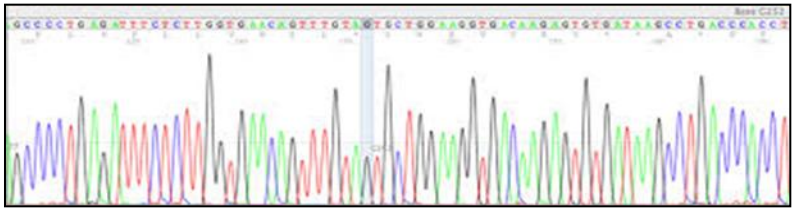
RNA extraction



RT-PCR with specific primers



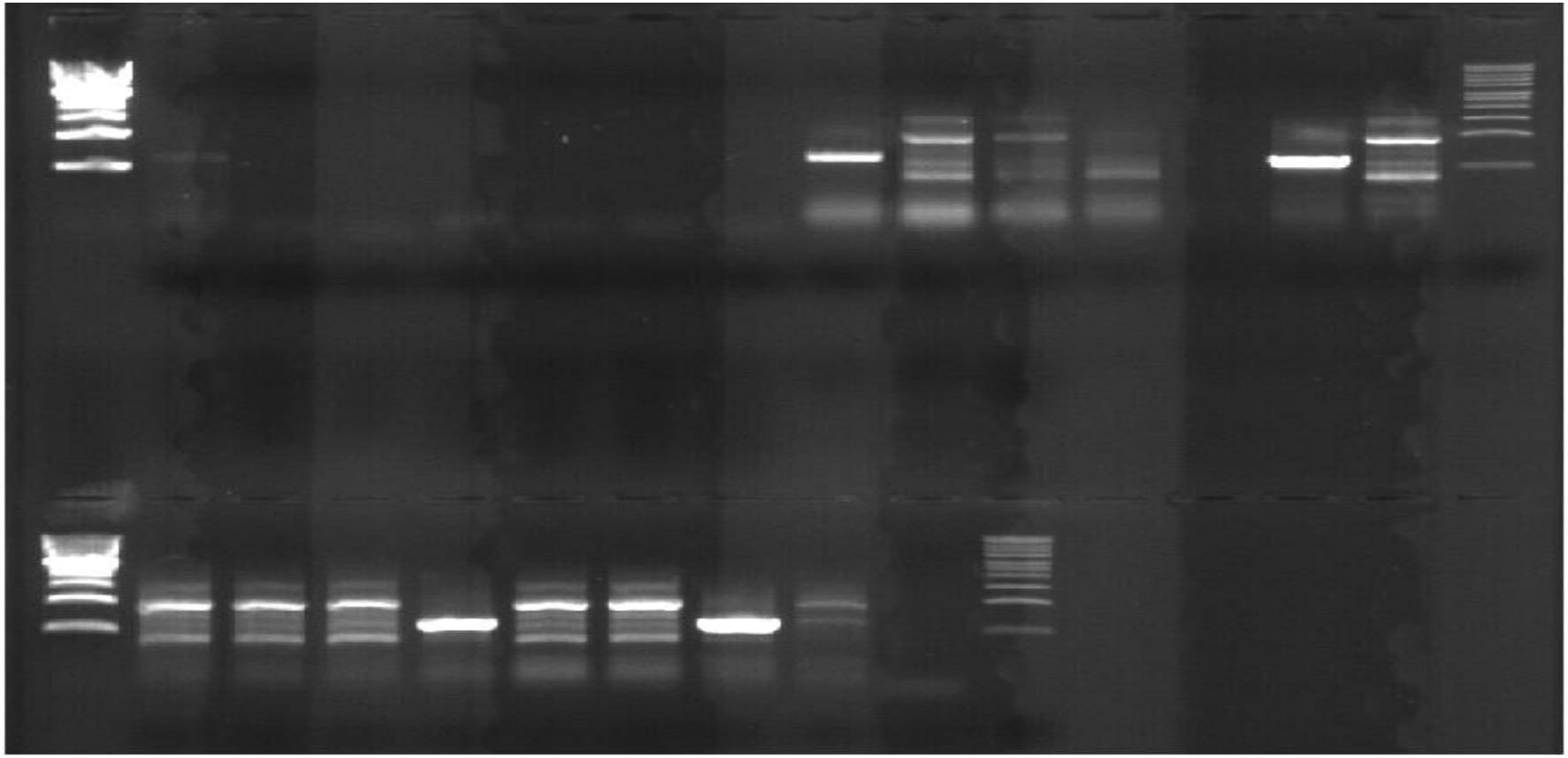
partial sequencing



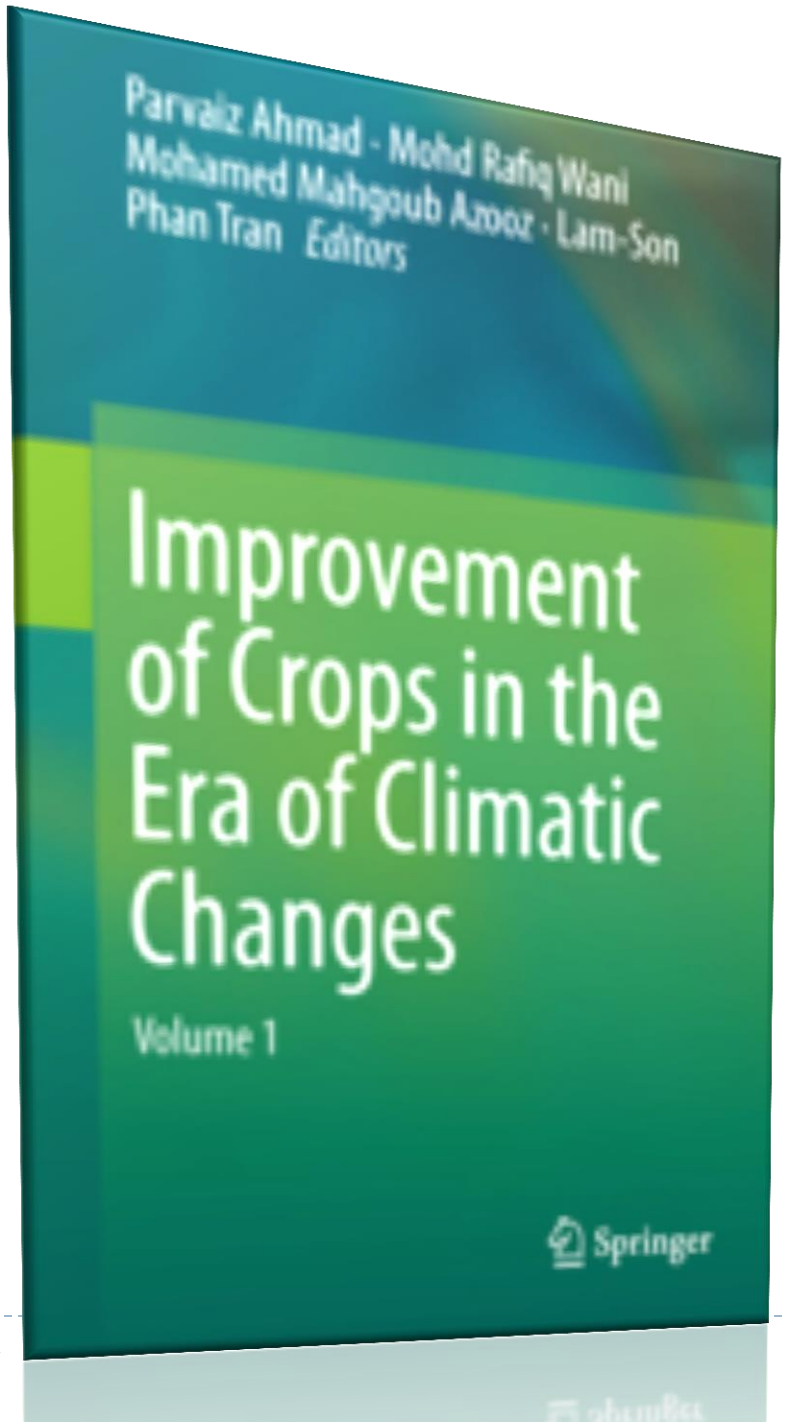
➔Sequence comparison with worldwide isolates

Result of RT-PCR analysis

ZYMV mlu-8800-3'/ xho-8200-5'



We detect ZYMV virus in melon samples by 3 methods too (immunostrips, ELISA and RT-PCR).



Biotic Stress and Crop Improvement

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Abstract

Biotic stress is one of the major environmental factors affecting plants. Viruses, fungi, bacteria, weeds, insects and other pests and pathogens represent a major constraint to agricultural productivity and a serious threat for vegetable and grain crops. Plant protection against pathogens and pests is a commercially important issue and one of the main directions of researches. Almost half of new infectious diseases identified in plants during the last 10 years have a viral nature. The number and distribution areas of some plant viruses in Europe have been increasing rapidly during the last 35 years that caused big problems from an economic point of view. Viral diseases have also become a real threat for different cultivars of vegetables, grains and other agricultural crops in our country. It causes extensive leaf yellowing, stem and leaf deformation, reducing the fruit quality, substantial yield loss and shortening the lifecycle of crops. The probable cause of decay of virus infected plants is not



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Research article

Histochemical visualization of ROS and antioxidant response to viral infections of vegetable crops grown in Azerbaijan

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ABSTRACT

Extremes of environmental conditions, such as biotic stresses, strongly affect plant growth and development and may adversely affect photosynthetic process. Virus infection is especially problematic in crops, because unlike other diseases, its impact cannot be reduced by phytosanitary treatments. The vegetable crops (*Solanum lycopersicum* L, *Cucurbita melo* L., *Cucumis sativus* L., *Piper longum* L., *Solánum melongéna* L., *Vicia faba* L.) showing virus-like symptoms were collected from fields located in the main crop production provinces of Azerbaijan. Infection of the plants were confirmed by Enzyme-linked immunosorbent assay using commercial kits for the following viruses: *Tomato yellow leaf curl virus*, *Tomato mosaic virus*, *Tomato chlorosis virus*, *Melon necrotic spot virus* and *Cucumber mosaic virus*, *Bean common mosaic virus* and *Bean yellow mosaic virus*. Generation sites of superoxide and hydrogen peroxide radicals and activities of enzymes involved in the detoxification of reactive oxygen species (catalase, glutathione reductase, ascorbate peroxidase, guaiacol peroxidase and superoxide dismutase) were examined in uninfected leaves and in leaves infected with viruses. High accumulation of superoxide and hydrogen peroxide radicals was visualized in infected leaves as a purple discoloration of nitro blue tetrazolium and 3,3′-diaminobenzidine tetrahydrochloride. It was found that the activities of APX and CAT significantly increased in all infected samples compared with non-infected ones. Dynamics of GR and Cu/Zn-SOD activities differed from those of CAT and APX, and slightly increased in stressed samples. Electrophoretic mobility profiling of APX, GPX and CAT isoenzymes was also studied.

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1. Introduction

Viral infections of crops cause significant losses in crop yield degrade the quality of agricultural products and are increasingly causing serious threat to food security. Viral infections may spread in many regions of the world that is an economically important problem and applied to all food, feed and industrial and horticultural crops grown in any region of the world, and is particularly relevant for vegetatively propagated plants, as a progressive accumulation of viruses in several generations' leads to a complete infection and degeneration of cultivars. It causes extensive leaf yellowing, stem and leaf deformation, reduced fruit quality, shortening the life-span and substantial crop loss of vegetables (Atkinson and Urwin, 2012; Britton et al., 2010). The annual losses caused by viruses of the same culture in a particular region are often expressed in terms of hundreds of millions and billions of dollars (Dangl and Jones, 2001; Duan et al., 2012; Zaitlin and Palukaitis, 2000). It is important therefore to understand and eventually enhance the natural response of plants to viral attack. When a plant recognizes an invading pathogen, active defense mechanisms are induced which can include a hypersensitive

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