FRONTIER LABORATORY

THE HOME OF QUALITY MICROPROPAGATED PLANTS

FRONTIER LABORATORY CC

"MICROPROPAGATION – AT THE CUTTING EDGE"

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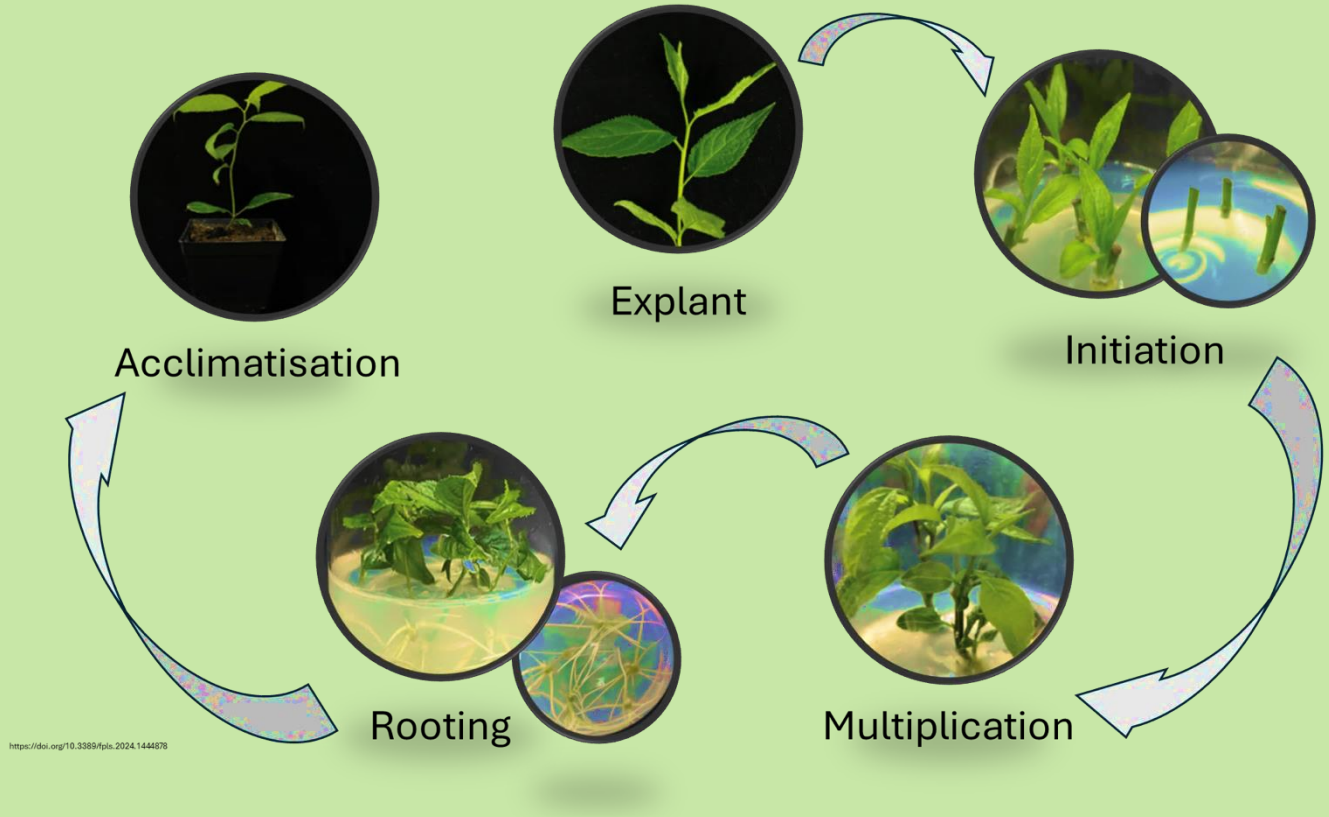
Dr. Adelle Roux

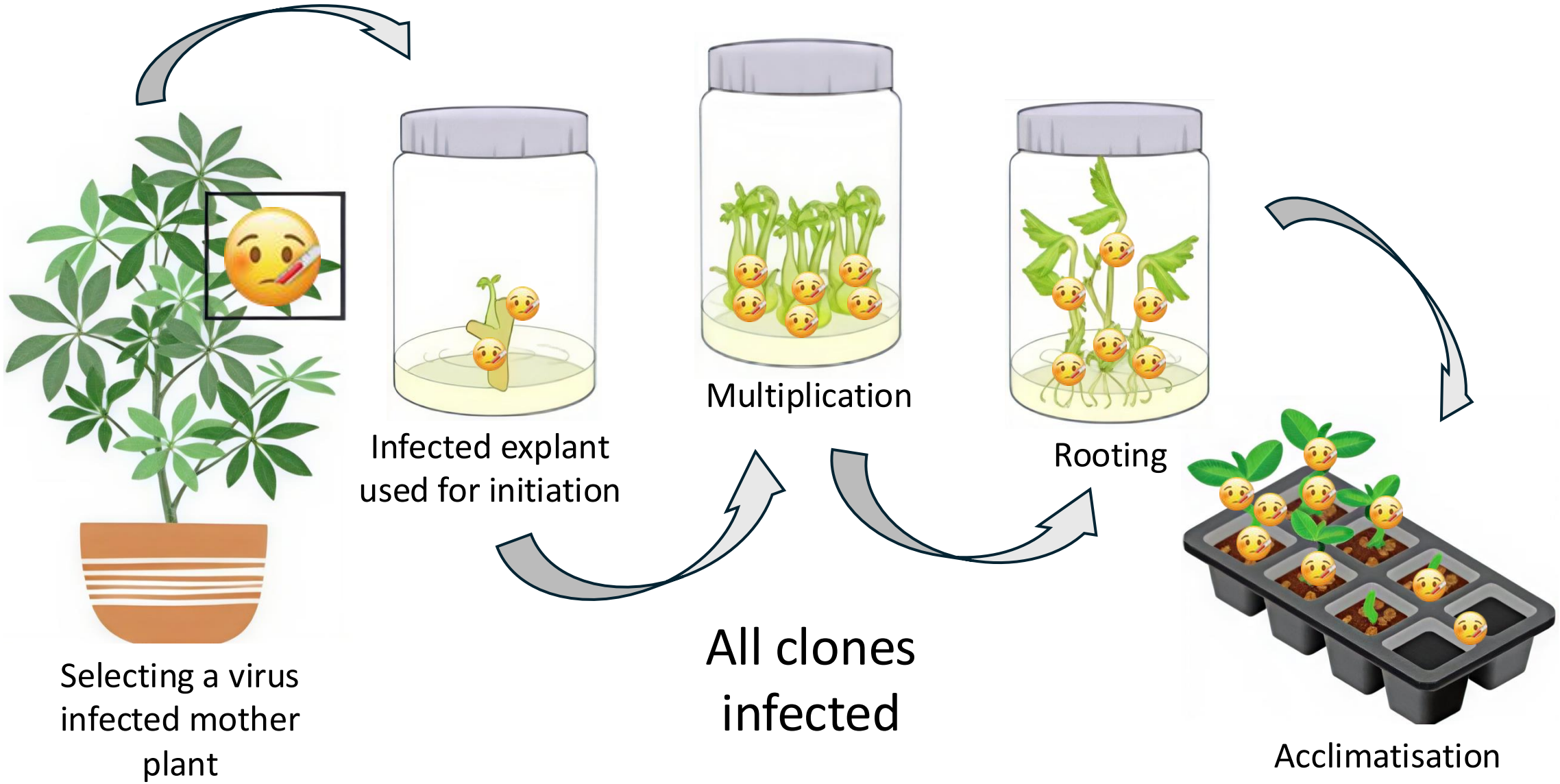
Virology in Micropropagation

Virology in Micropropagation

- **Micropropagation:** Producing *clones* from a *single plant* (mother plant) in a *sterile* environment (lab)
- **Five steps:**
  - ❑Mother plant selection
  - ❑Explant initiation
  - ❑Multiplication
  - ❑Rooting
  - ❑Acclimatisation

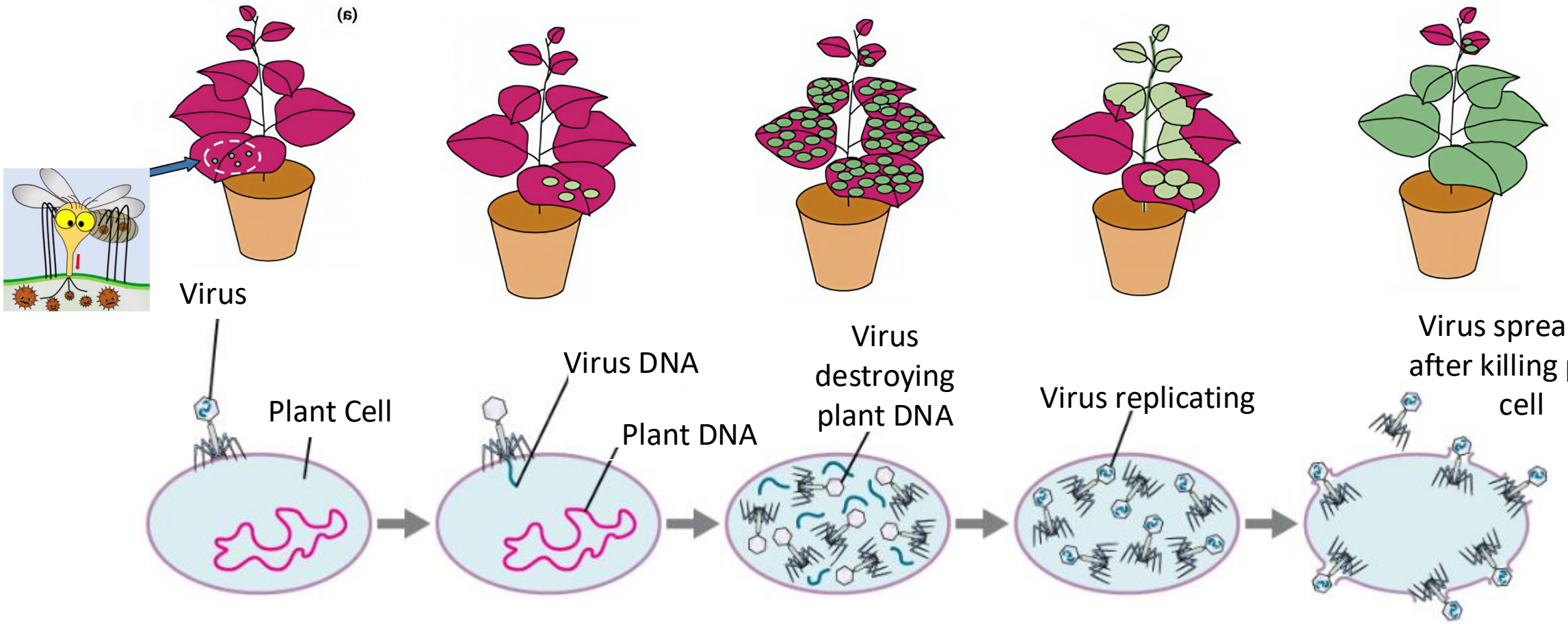


Importance of selecting a virus-free mother plant



What is a plant virus and how do they spread?

Sub-microscopic, infectious, non-living “particle” that hijacks a plant’s own cells to reproduce, using the plant's resources to create more virus particles



https://ecampusontario.pressbooks.pub/microbio/chapter/the-viral-life-cycle/

What is a plant virus and why are they so dangerous?

Devastating effects on yield and quality, leading to extensive financial losses

Crop	**Country**	**Virus**	**Losses**
Banana	Africa, Asia, India	Banana bunchy top virus	100% yield loss
Cassava	Africa, India	African cassava mosaic virus	$3 billion
Maize	Africa, Asia, South America	Maize chlorotic mottle virus	$52 million
Potato	US	Potato Virus Y	$100 million
Potato	UK	Potato leafroll virus	$50 million
Barley	UK	Barley yellow dwarf virus	$14 million
Rice	**India**	**Rice tungro virus**	**$1.5 billion**
Cacao	Africa	Cacao swollen shoot virus	200 million trees
Stone fruits	Europe, Asia, America	Plum pox virus	100% yield loss

Top ten most important plant viruses

International Committee on Taxonomy of Viruses (ICTV)

- *Tobacco Mosaic Virus* (TMV)
- *Tomato Spotted Wilt Virus* (TSWV)
- *Tomato Yellow Leaf Curl Virus* (TYLCV)
- *Cucumber Mosaic Virus* (CMV)
- *Potato Virus Y* (PVY)
- *Cauliflower Mosaic Virus* (CaMV)
- *African Cassava Mosaic Virus* (ACMV)
- *Plum Pox Virus* (PPV)
- *Brome Mosaic Virus* (BMV)
- *Potato Virus X* (PVX)

Tobacco mosaic virus (TMV)

- ❑ The first virus ever discovered
- ❑ Infects over 350 species
  - Tomato, Pepper, Potato, Cannabis, Petunia, Impatiens, Chrysanthemum, Geranium, Begonia, Verbena
- ❑ Transmitted via
  - vectors (thrips)
  - contaminated tools
  - plant debris, surviving years in dead tissue
- ❑ Causing
  - mottled, mosaic-patterned leaves,
  - stunted growth
  - reduced yields

Tomato spotted wilt virus (TSWV)

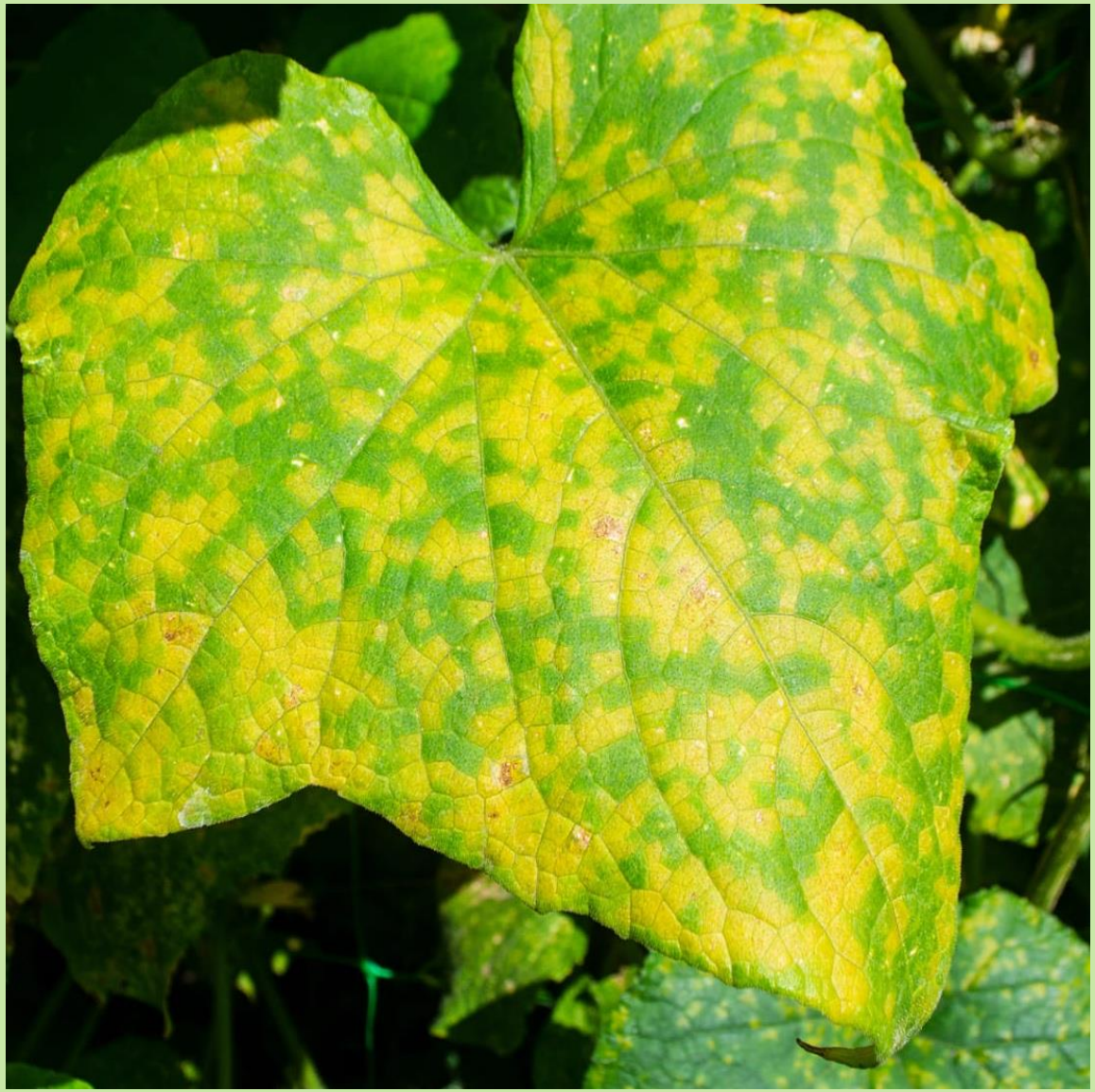
- ❑ Tomato and Peppers
- ❑ Transmitted by thrips
- ❑ Bull's eye pattern on fruit and leaves
- ❑ Necrosis
- ❑ Stunting
- ❑ Deformed leaves and fruit
- ❑ Causes huge losses in vegetables

Tomato Yellow Leaf Curl Virus (TYLCV)

- ❑ Transmitted by whiteflies
- ❑ Leaf Curling ("cup" shape)
- ❑ Yellowing of leaves
- ❑ Deformed fruit

Cucumber Mosaic Virus (CMV)

- ❑ Infects more than 1000 species
- ❑ Warty and misshapen fruit (“white pickle”)
- ❑ Stunting and reduced size of fruit
- ❑ Internal quality issues
- ❑ Flavor alterations (bitter taste)



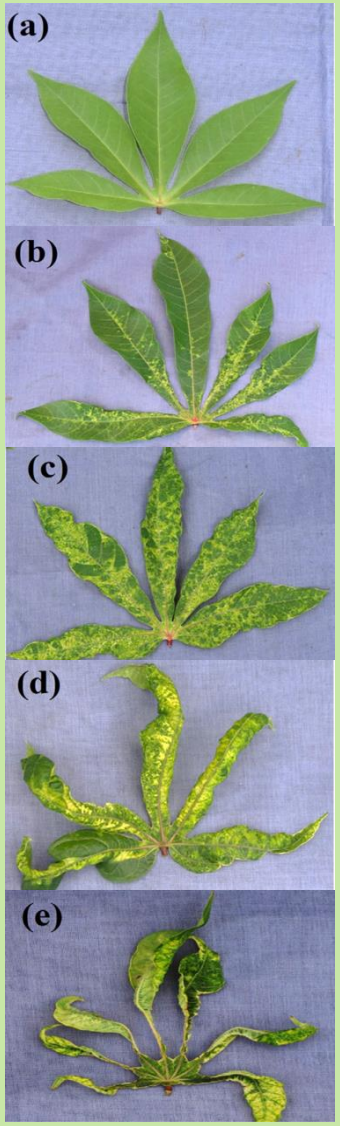
Potato Virus Y (PVY)

- ❑ Infects tomato, tobacco and peppers
- ❑ Mosaic patterns on leaves
- ❑ Stunted growth
- ❑ Leaf necrosis
- ❑ Tuber necrotic rings

Cauliflower Mosaic Virus (CaMV)

- ❑Vein clearing (translucent vein)
- ❑Internal spotting
- ❑Leaf deformaties
- ❑Necrosis
- ❑Reduced yield
- ❑Stunting

African Cassava Mosaic Virus (ACMV)

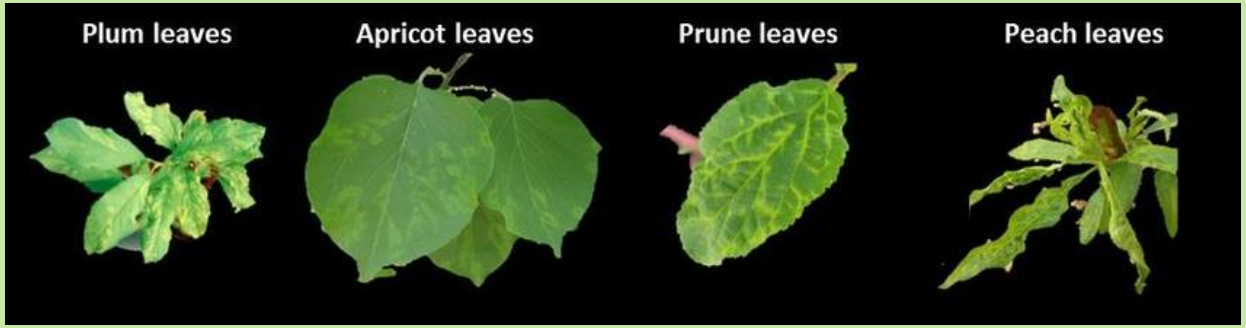


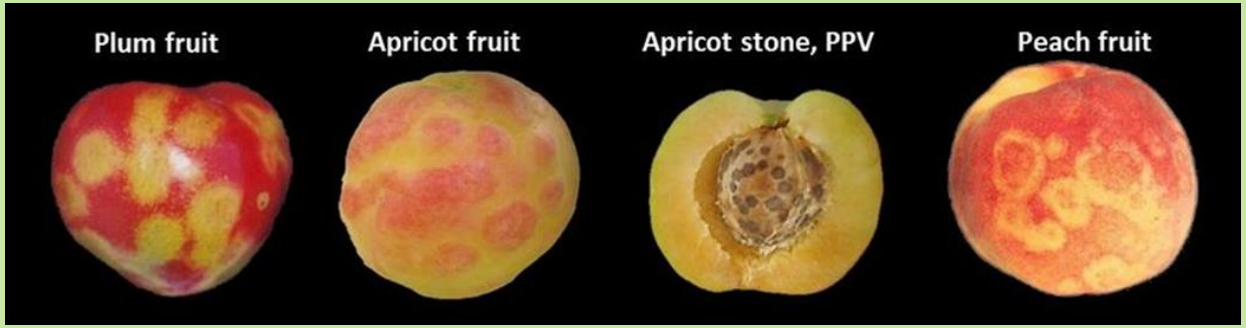
- ❑ Leaf mosaic patterns
- ❑ Leaf distortion
- ❑ Leaf narrowing
- ❑ Stunted growth
- ❑ Reduced yield
- ❑ Premature defoliation
- ❑ S-shaped leaf stalks

"Candlestick" Syndrome: thickened stems that may turn purple, extreme reduction in leaf size, foliage so limited that the plant resembles a "candlestick".

Plum Pox Virus (PPV)

- The most serious viral disease of **stone fruits**
- Reddish rings/spots on fruit
- Deformed leaves and fruit
- Premature fruit drop
- Reduced sugar content



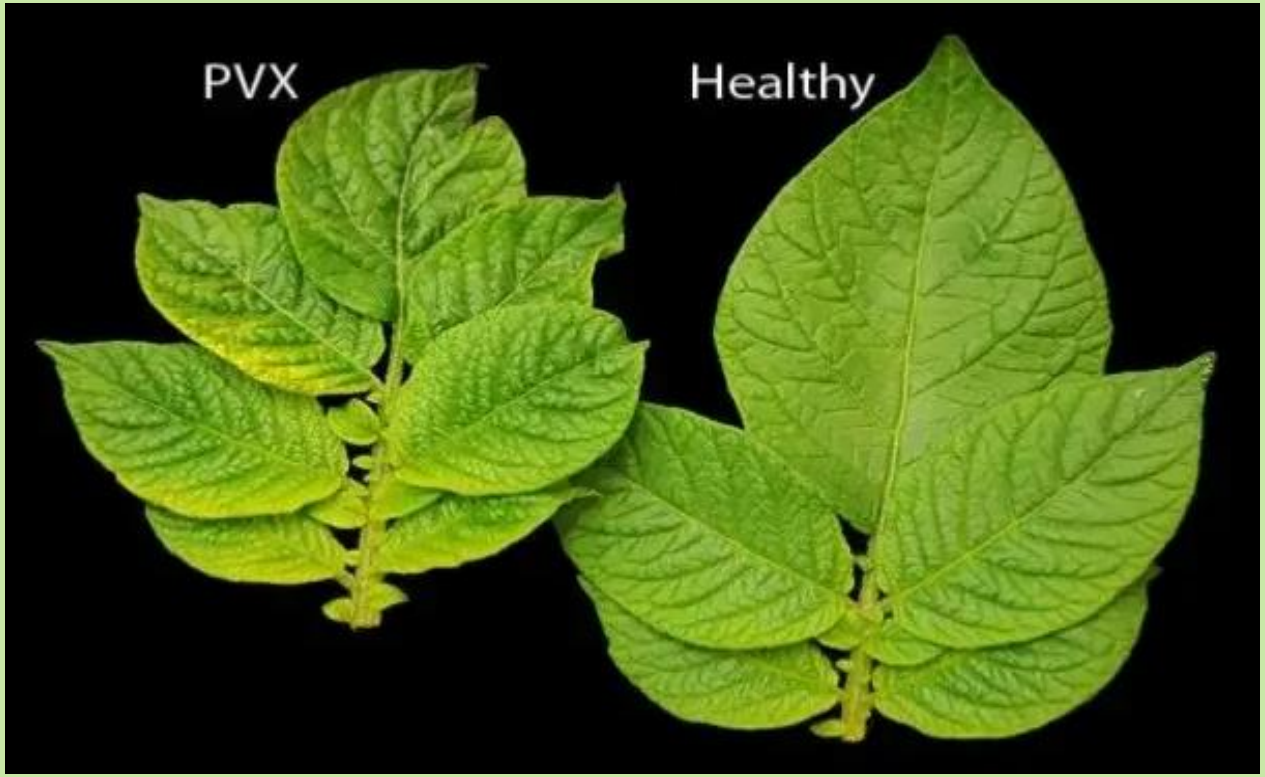


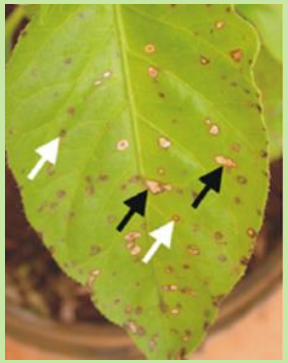
Brome Mosaic Virus (BMV)

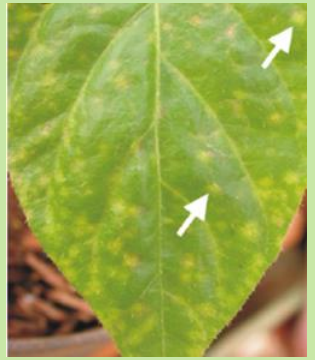
- ❑ Shrivelled leaves and chlorosis
- ❑ Stunting
- ❑ Quinoa
- ❑ Soybean
- ❑ Maize
- ❑ Wheat

Potato Virus X (PVX)

- ❑ Not severe symptoms
- ❑ Leaf crinkling
- ❑ Low tuber quality
- ❑ Co-infections
- ❑ Combined with PVX
- ❑ Severe symptoms

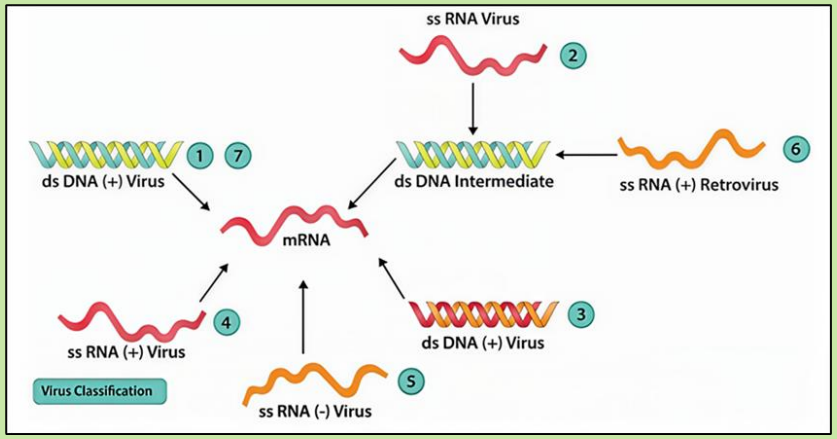


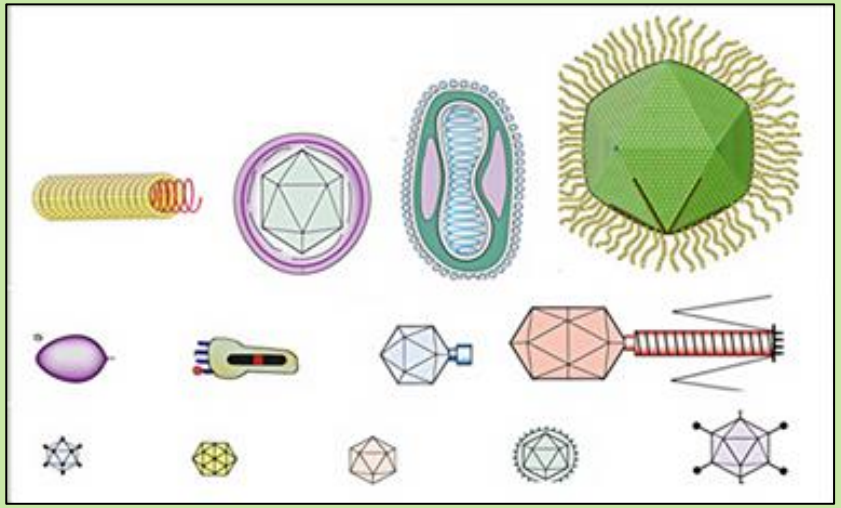


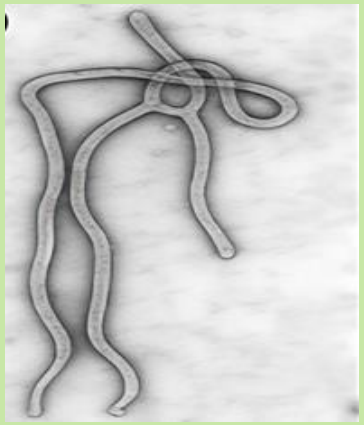


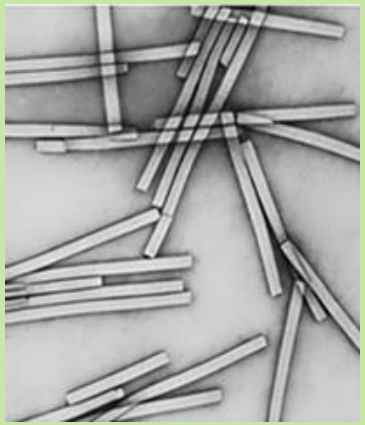
What is a virus?

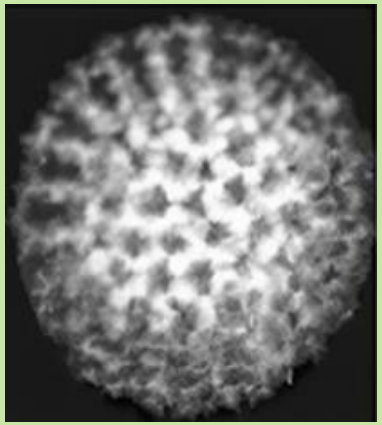
- ❑ Sub-microscopic non-living “particle”
- ❑ Classified according to genetic structure
- ❑ Building blocks – DNA/RNA
- ❑ dsDNA or ssDNA and dsRNA or ssRNA
- ❑ Structure determines shape
- ❑ Helical
- ❑ Filaments
- ❑ Rigid Rods
- ❑ Bacilliform
- ❑ Spherical





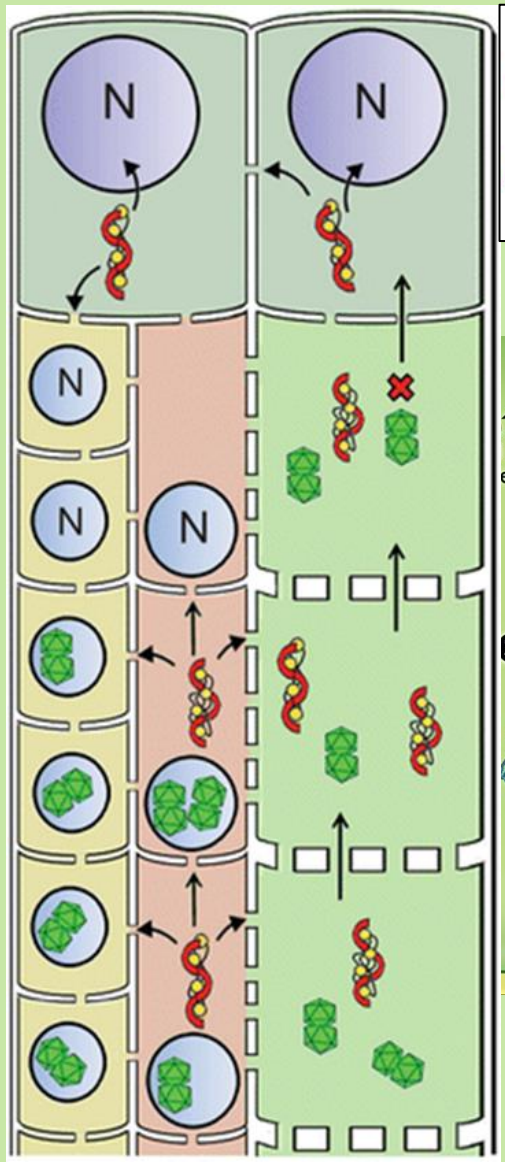


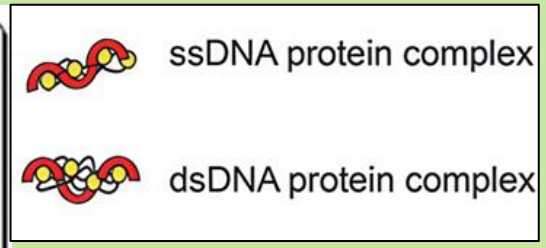


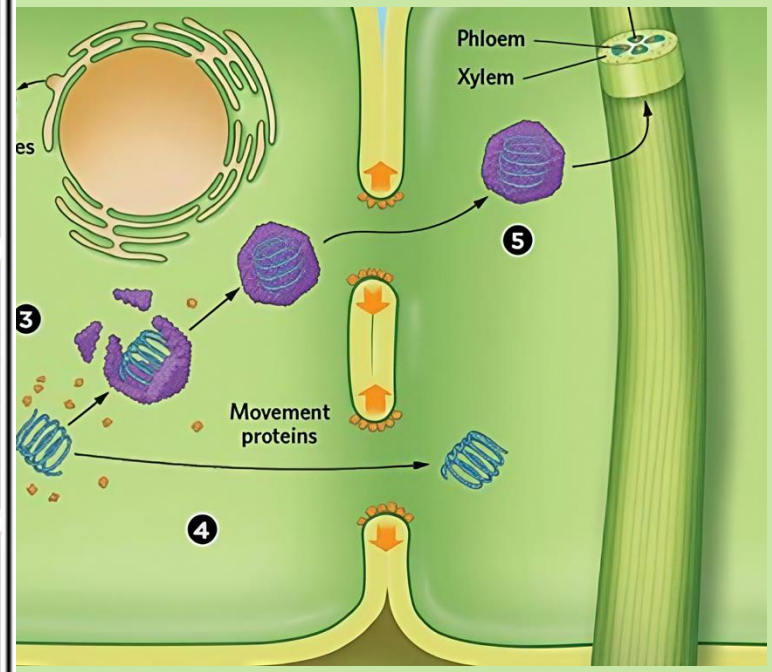


Why is the shape and size of the virus important?

- ❑ Influence movement
- ❑ Long, filamentous viruses move slowly
- ❑ Small rod-shaped viruses spread rapidly
- ❑ Viruses spread through the plant via:
- ❑ Cell-to-cell (local) movement
  - Viruses move between adjacent cells through microscopic channels that connect the plant cells
- ❑ Systemic (long-distance) movement
  - Virus reaches the vascular tissue and moves rapidly through the phloem of the plant, reaching the roots, young leaves and fruits





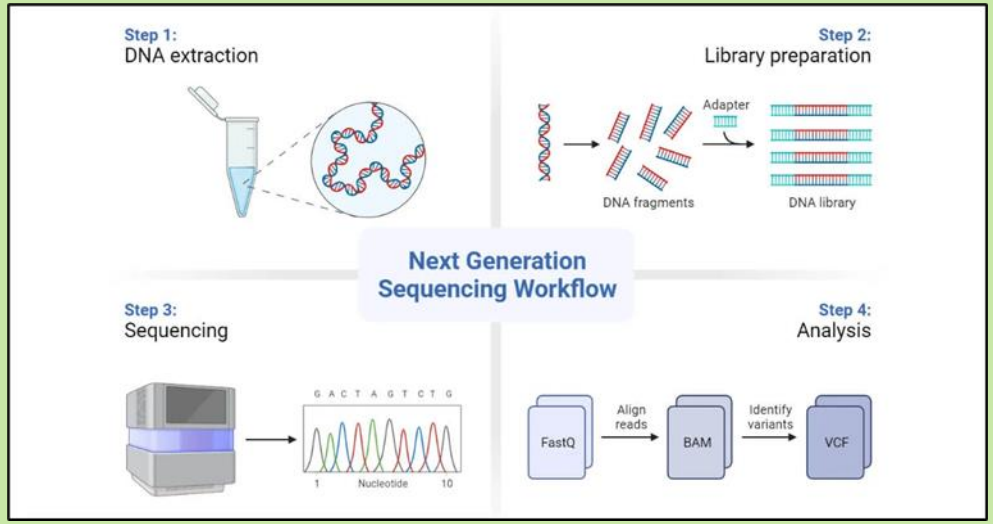


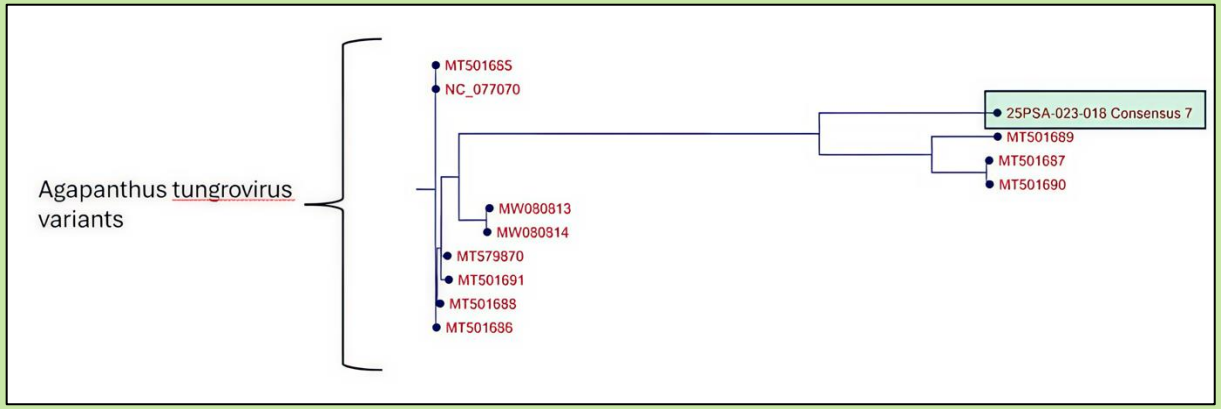
How do we identify viruses?

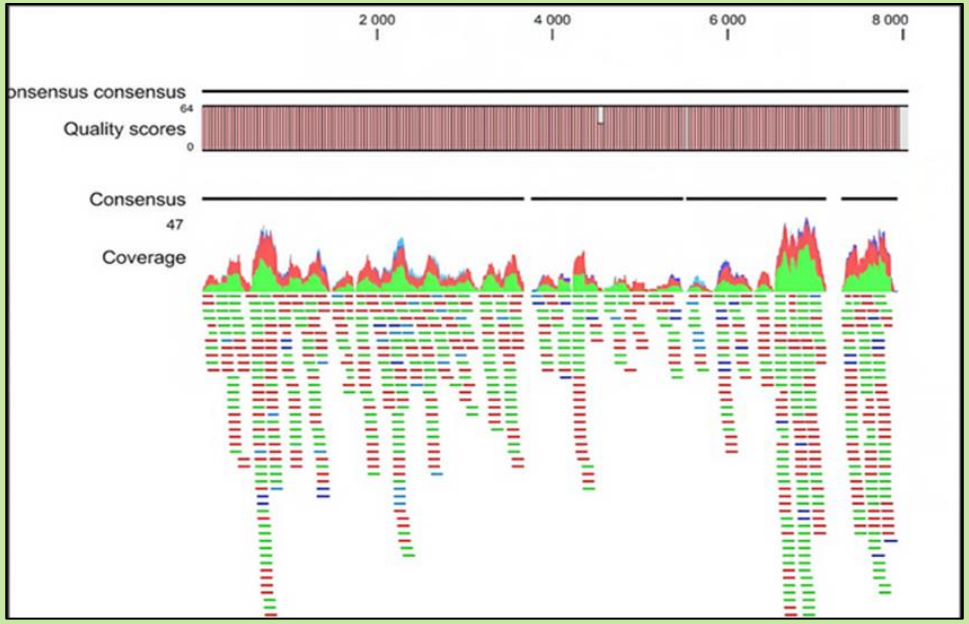
- **Serological Methods (Antibody-Based):** These detect the coat protein of the virus and are highly effective for routine screening.
- **Molecular Techniques (Nucleic Acid-Based):** These methods detect the viral RNA or DNA, offering high sensitivity and precision.
- **Electron Microscopy:** This is where the physical structure of virus particles is visualised under a specialised microscope.
- **Next-Generation Sequencing (NGS):** Enables comprehensive detection, allowing identification of known, unknown, and multiple viruses simultaneously.

Next-Generation Sequencing (NGS)

- ❑ Determine exact order of DNA/RNA of virus
- ❑ Sequence millions of fragments simultaneously
- ❑ Identify all viruses in plant in one single test
- ❑ Correlated with databases
- ❑ Novel viruses







How to get rid of a plant virus: Virus Elimination

Meristem culture	• Advantages: Most of the virus particles will not penetrate into the meristem • Disadvantages: Meristem excision is tedious and requires skilled labour
Chemotherapy	• Advantages: Antiviral chemicals are effective in disrupting viral replication • Disadvantages: High concentrations induce abnormalities or cell death
Thermotherapy	• Advantages: Deactivation and rupture of viral particles • Disadvantages: Higher temperatures reduce plant survival
Cryotherapy	• Advantages: Requires no specialised equipment or costly chemicals • Disadvantages: May damage healthy cells
Electrotherapy	• Advantages: Reduces virus virulence by degrading nucleoproteins • Disadvantages: Costly and shoot may exhibit abnormalities

Thermotherapy

- Virus DNA/RNA “degrades” at high temperatures (35-54 ℃)
- Explants temperatures for a certain period of time

Chemotherapy

- Chemicals (viricides) prevent virus replication
- Ribavirin - treatment of viral infections in humans such as chronic hepatitis C

Cryotherapy

- Freeze explant in liquid nitrogen
- Virus DNA/RNA will degrade

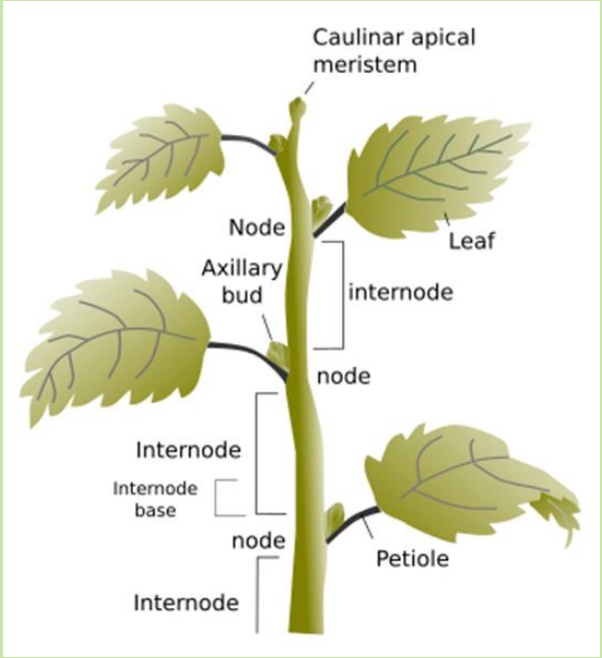
Electrotherapy

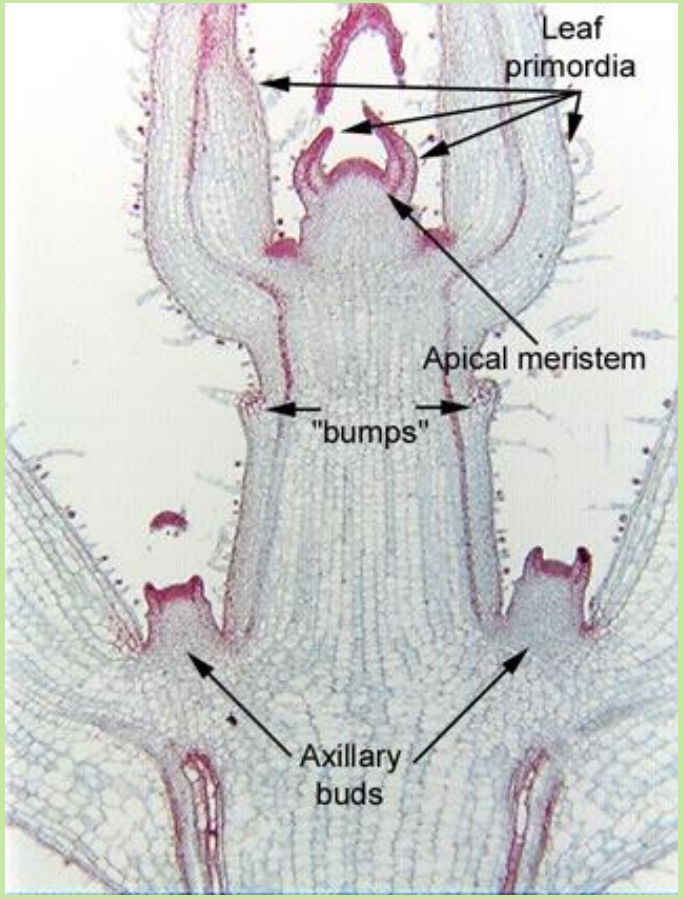
- Applying a controlled electric current
- Virus proteins disrupted

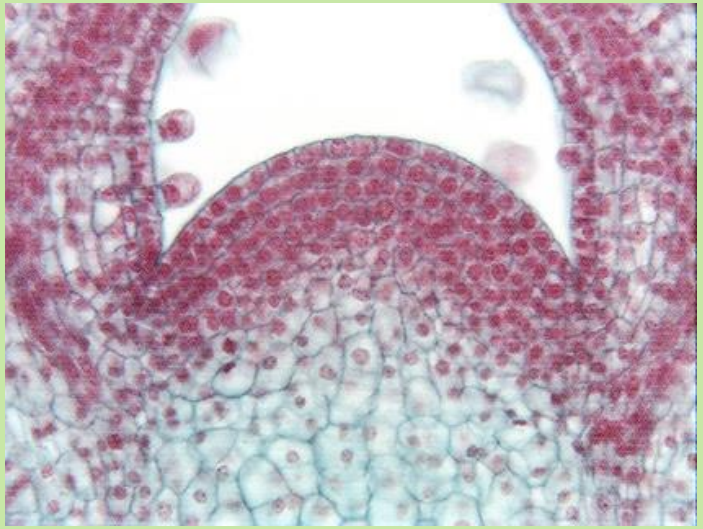
Meristem culture

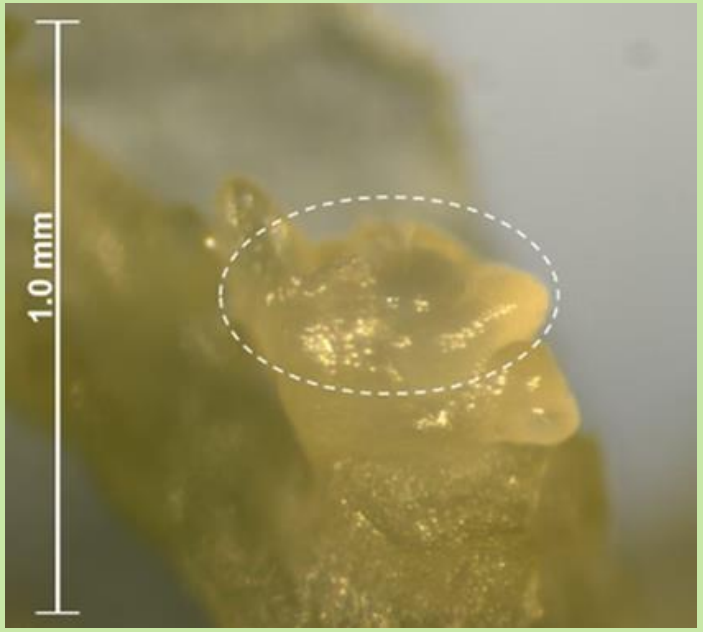
What is the meristem

- Meristems (growth tips)
  - Apical dome (AD)
  - Leaf primordia (LP)
- Actively dividing
- Undifferentiated cells





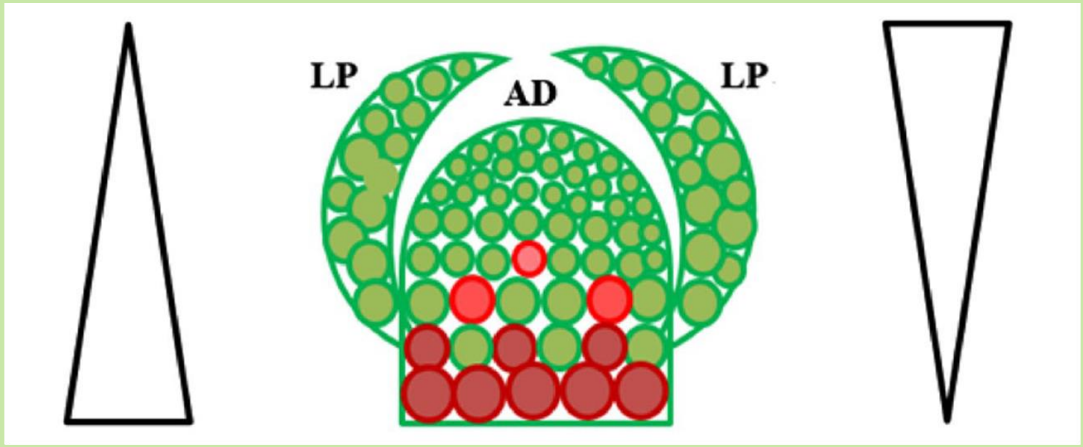




Meristem culture

- ❑ Meristems considered virus-free
- ❑ Cells divide faster than virus replicates
- ❑ No vascular connection between meristem and rest of plant
- ❑ Virus unable to reach the cells at the tip

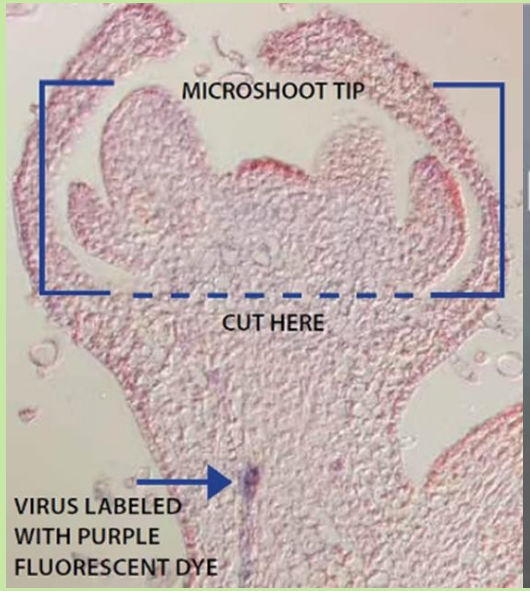
- ❑ Meristem sizes differ between crops
- ❑ Usually about 0.3 to 0.5 mm
- ❑ Mostly effective against long phloem-based viruses
- ❑ Combined with other therapies to eliminate smaller viruses

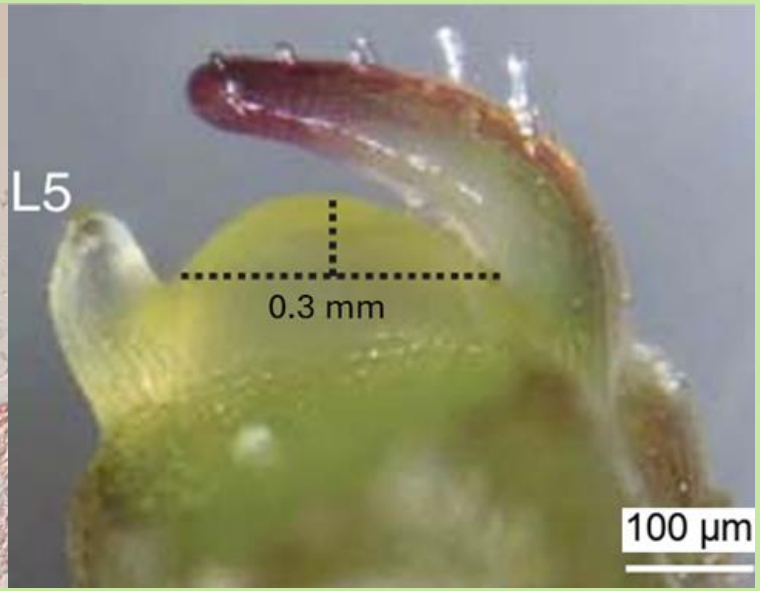


Shoot regeneration %

Virus infected shoot tip

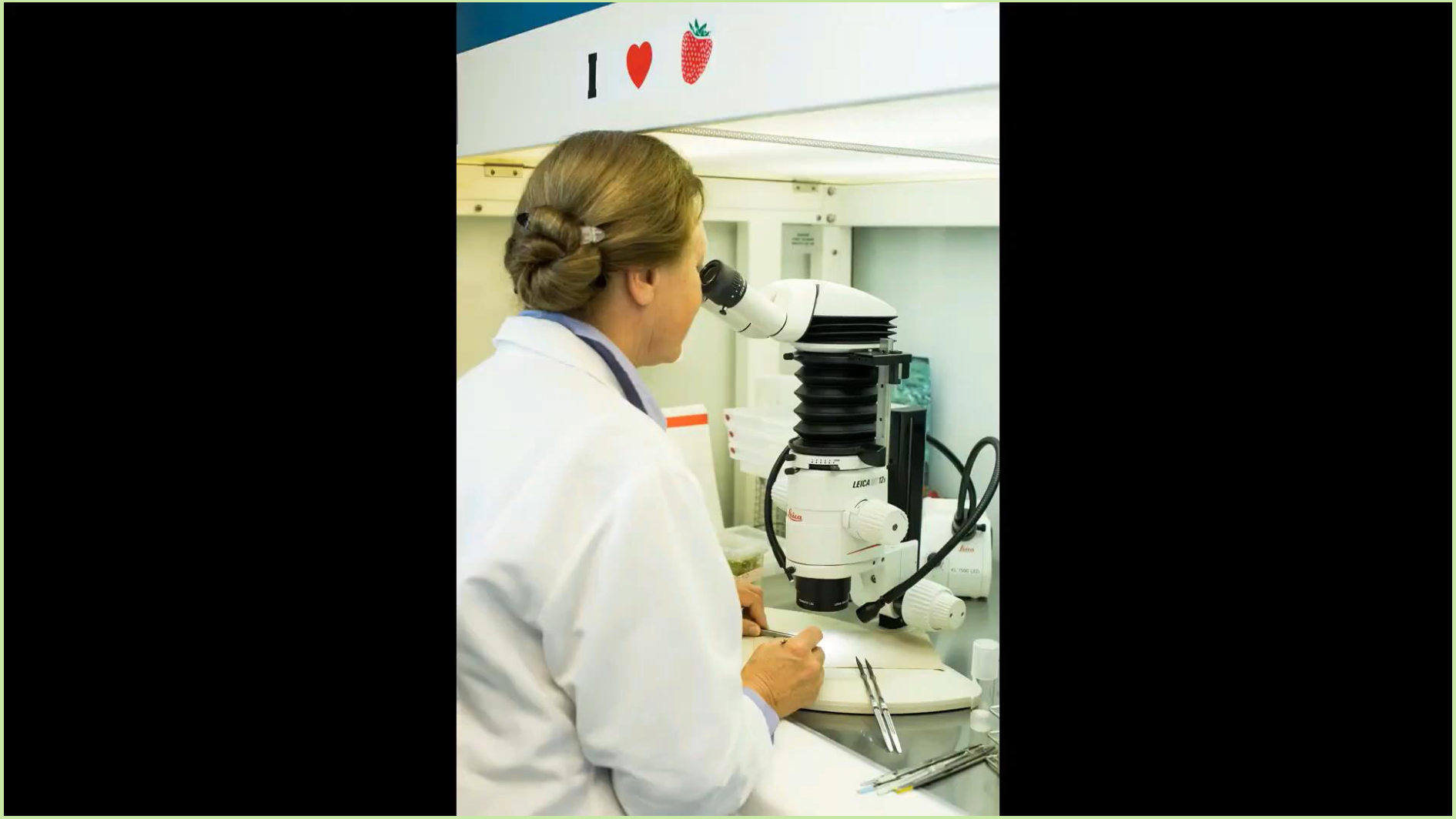
Virus-free frequency %





Grapevine Meristem Excision

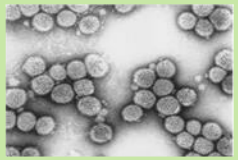
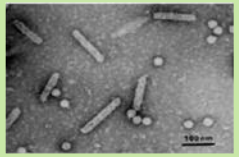
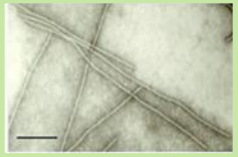
Foundation Plant Services, University of California UCD, Narrator: Susan Sim, Technician: Waclawa Pudlo, Videographer: Nihn Khuu



What has been found in Agapanthus spp.

Plant virus present	Year	Country	Description of symptoms
Nerine Virus X	1980	England	Chlorotic mottle symptoms
	2006	Japan	
	2010	Netherlands	
	2011	Taiwan	
	2021	New Zealand	
Tomato Spotted Wilt Virus	2000	Tasmania	**Severe stunting**. **Failure to flower**. Premature senesced. Abnormal leave growth. Death.
	2023	South Africa	
	2024	China	
Eggplant Mottled Dwarf Virus	2014	Italy	Bright white and yellow rectangular mosaic, oval to linear patches or stripes on leaves, flower stalks and buds
Agapanthus Tungro Virus	2021	South Africa	Mottling, Chlorosis, Streaks, Necrosis
Agapanthus Velari Virus	2021	South Africa	Mottling, Chlorosis, Stunting
Agapanthus Virus A	2021	South Africa	Mottling, Chlorosis, Stunting, Ring spotting
Agapanthus Clara Virus B	2021	South Africa	Mottling, Chlorosis, Streaks
Nerine Latent Virus	2021	South Africa	Mild leave mottle

What do Agapanthus viruses look like?

Virus	Class	Shape and size	Transmitted	Susceptible Hosts	
Nerine Virus X	*Potexvirus*	Filamentous Length: 540nm	Mechanical	Amaryllidaceae, Nerine, Agapanthus	
Tomato Spotted Wilt Virus	*Orthotospovirus*	Spherical Diameter: 90nm	Vector	Periwinkle, Cucumis, Nicotiana, Petunia	
Eggplant Mottled Dwarf Virus	*Rhabdoviridae*	Bullet-shaped Diameter: 90nm	Mechanical	Solanum, Nicotiana, Quinoa, Cucumis	
Agapanthus Tungrovirus	*Caulimoviridae*	Bacilliform Width: **35nm**	Arthropods	Rice	
Agapanthus Velarivirus	*Closteroviridae*	Filamentous Length: **2200nm**	Grafting; Arthropods	Cordyline, Vitis, Prunus, Malus	
Nerine Latent Virus	*Betaflexiviridae*	Filamentous Length: 665nm	Mechanical; Arthropods	Nerine, Dahlia, Nicotiana, Datura	

How to Agapanthus viruses affect plants?

A

Tomato Spotted Wilt Virus **(TSWV)**

D

Agapanthus Velarivirus **(AgVV)**

E

Agapanthus Tungrovirus **(AgTV)**

F

Agapanthus Velarivirus **(AgVV)**

Agapanthus Tungrovirus **(AgTV)**

B

Eggplant Mottle Dwarf Virus **(EMDV)**

G

Nerine Latent Virus **(NeLV)**

H

Nerine Virus X **(NVX)**

I

Nerine Latent Virus **(NeLV)**

Nerine Virus X **(NVX)**

C

Agapanthus Claravirus B **(AgCVB)**

J

Agapanthus Velarivirus **(AgVV)**

Nerine Latent Virus **(NeLV)**

K

Agapanthus Velarivirus **(AgVV)**

Agapanthus Tungrovirus **(AgTV)**

Nerine Latent Virus **(NeLV)**

L

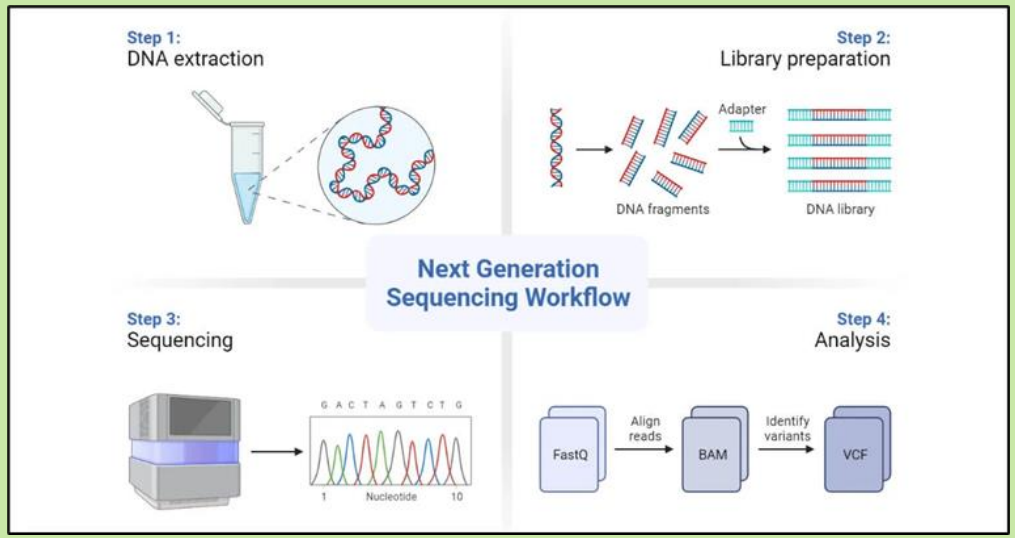
Agapanthus Velarivirus **(AgVV)**

Agapanthus Tungrovirus **(AgTV)**

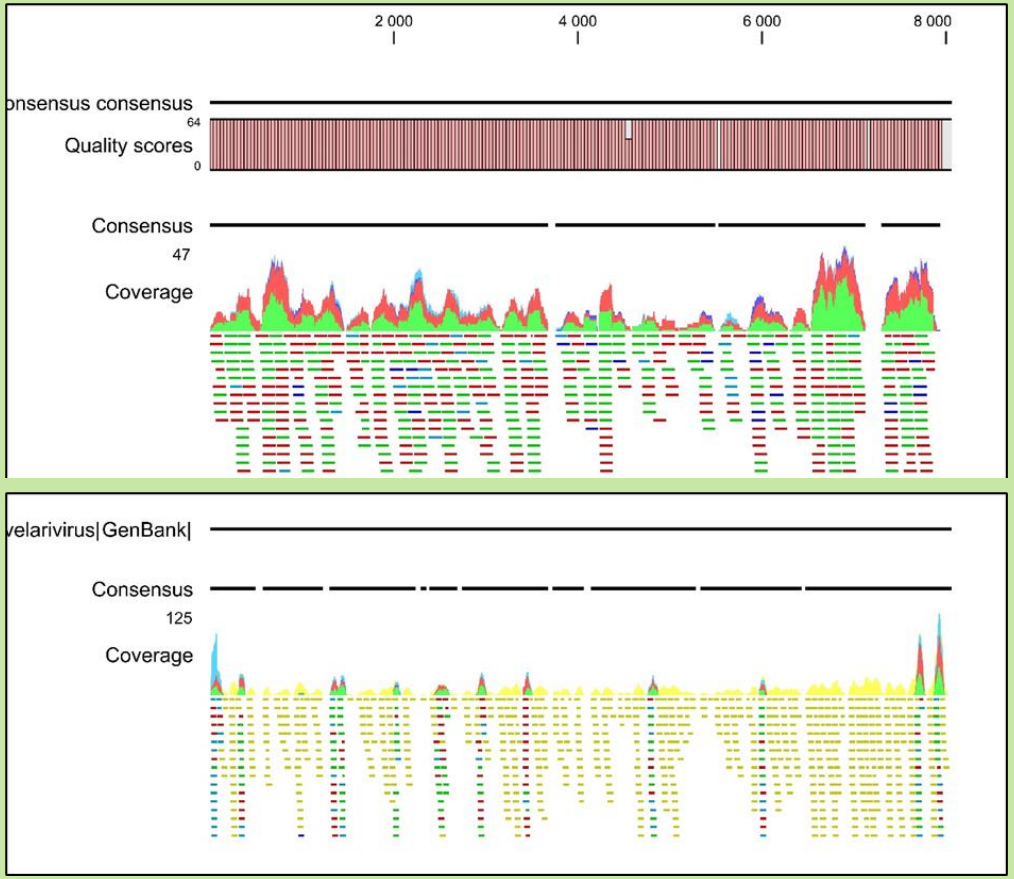
Agapanthus Virus A **(AgVA)**

What viruses are found in our Agapanthus spp.?

- ❑ Pilot study by Frontier Laboratory in 2025
- ❑ Determine virus status of stock plants
- ❑ Ten Agapanthus varieties selected
- ❑ Based on visual symptoms
- ❑ Genetic material of all the plants were pooled taken for NGS



- ❑ Results: only two viruses were present
- ❑ Agapanthus Tungrovirus (AgTV)
- ❑ Agapanthus Velarivirus (AgVV)



Virus detection in Agapanthus by Frontier Laboratory

- ❑ 6 Negative
- ❑ 3 AgTV
- ❑ 1 AgVV

❑ Tested each variety separately to see what viruses were present

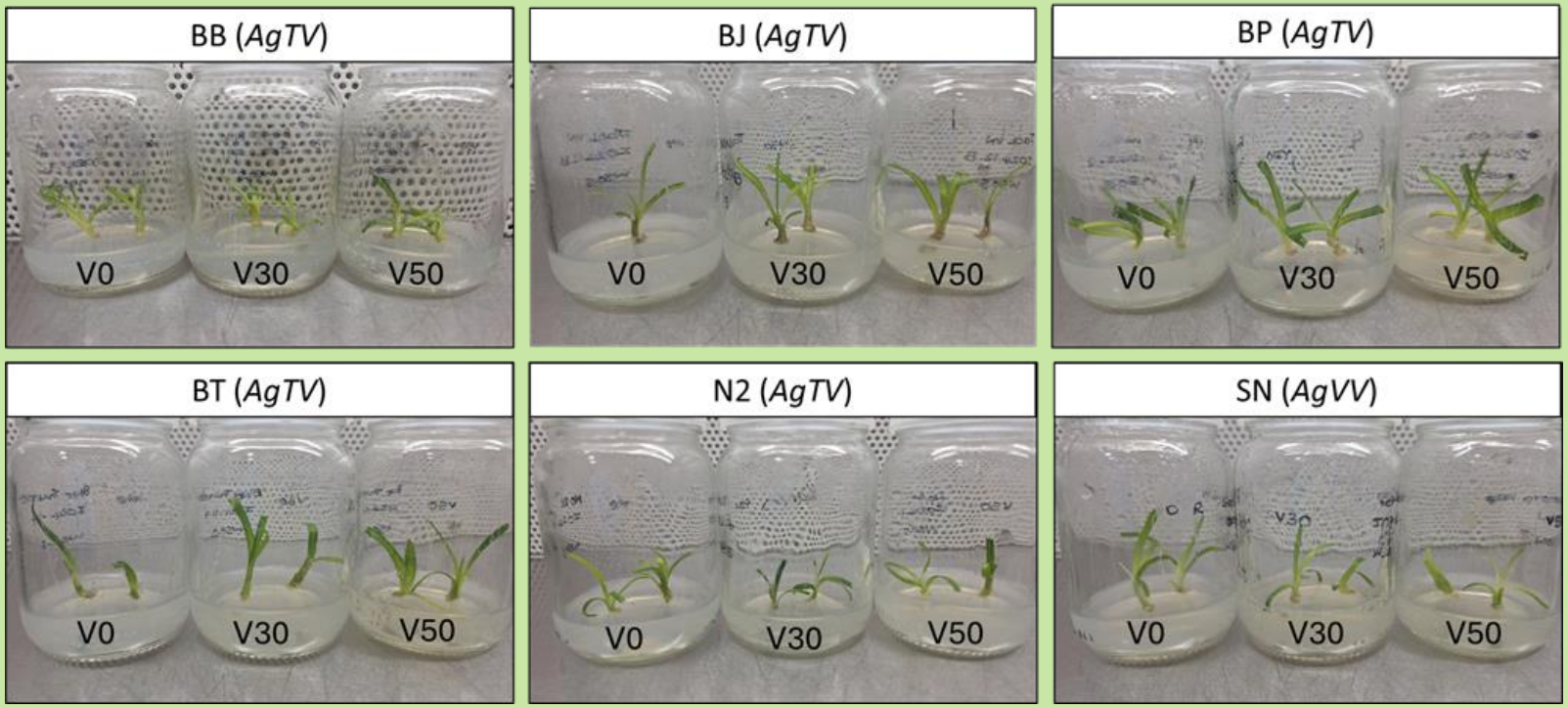
Virus detection in Agapanthus by Frontier Laboratory

- To expand on the study
- Tested another 40 varieties for these two viruses
- Eight out of 40 tested positive for AgTV (20%)
- Two out of 40 tested positive of AgVV (5%)
- Able to eliminate these viruses from the plants?
- Five AgTV – positive varieties
- One AgVV – positive variety

	Agapanthus Tungrovirus	**Agapanthus Velarivirus**	**Negative**
Tested 40 vaieties	8	2	30
	20%	5%	75%

Agapanthus Virus Elimination at Frontier Laboratory

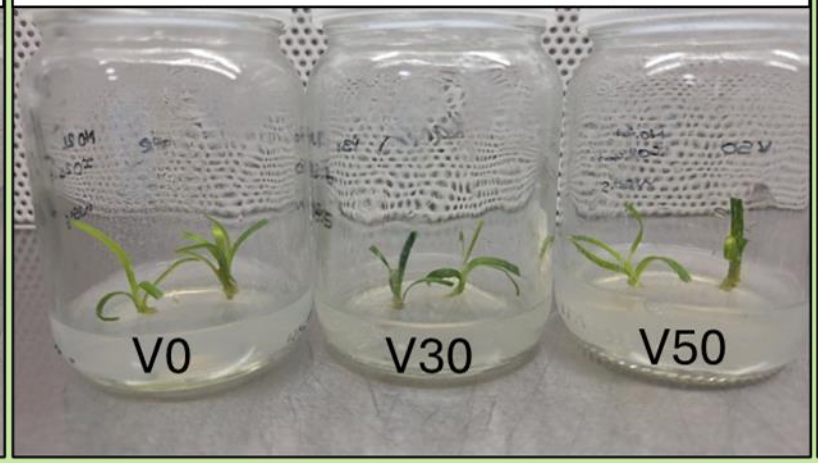
- ***Chemotherapy*** (Ribavirin as viricide)
- Viricide = phytotoxic (concentration and time)
- V0 = No viricide
- V30 = 30mg/L viricide
- V50 = 50mg/L viricide
- Grow 28 days on viricide
- Evaluate plant health
- ***Excise meristem***



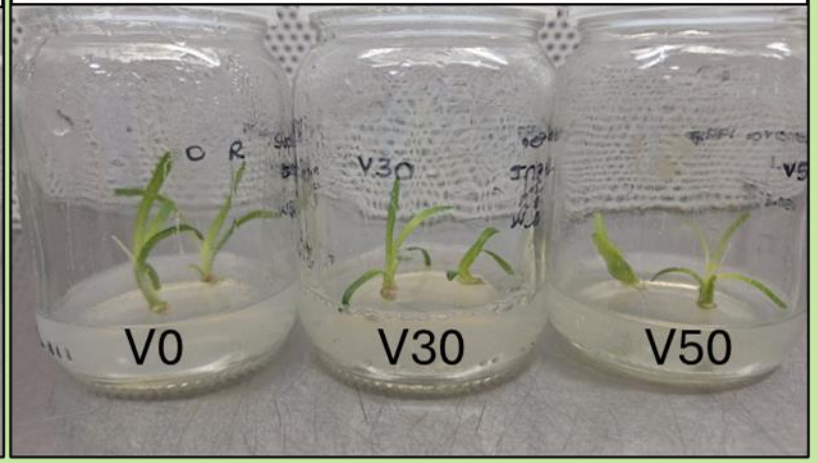
Agapanthus Chemotherapy Results after 28 days on viricide

BT-01 (*AgTV*)

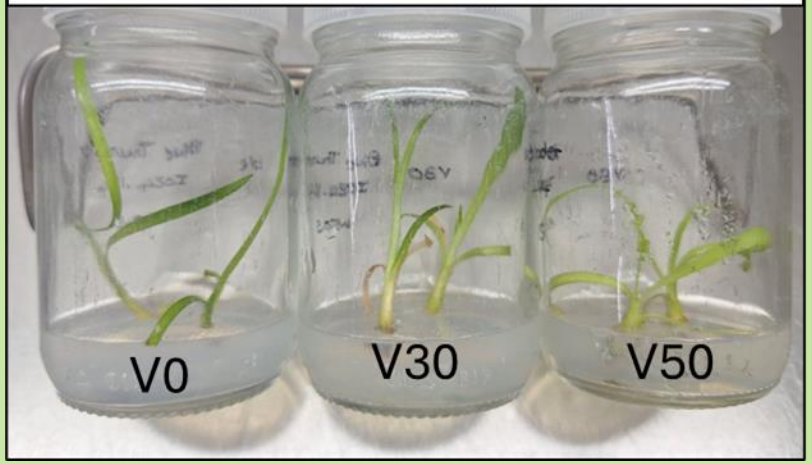
N2-01 (*AgTV*)



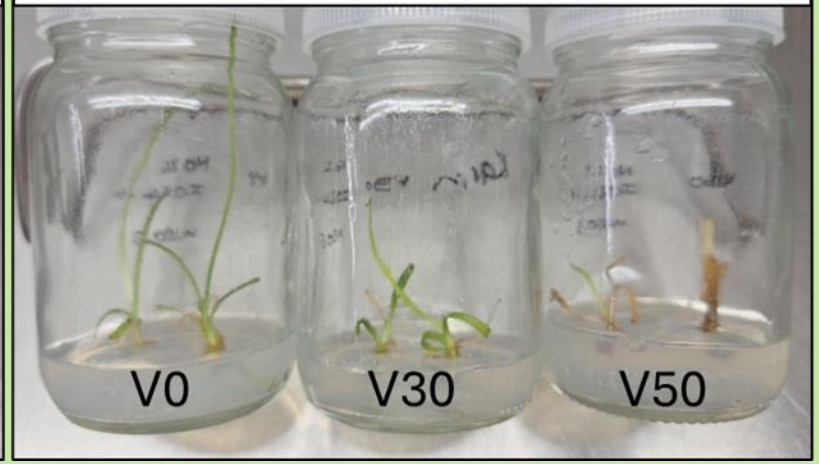
SN-01 (*AgVV*)



BT-28

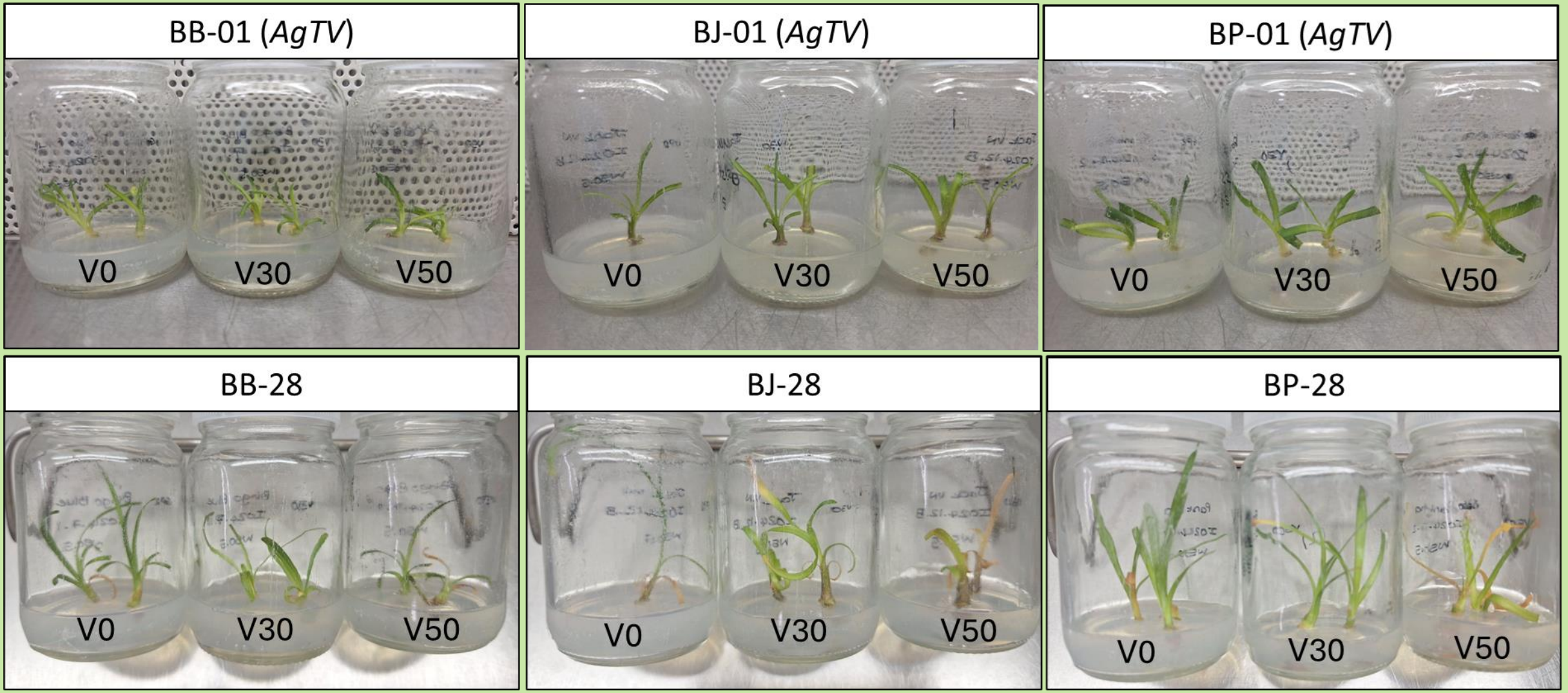


N2-28



SN-28

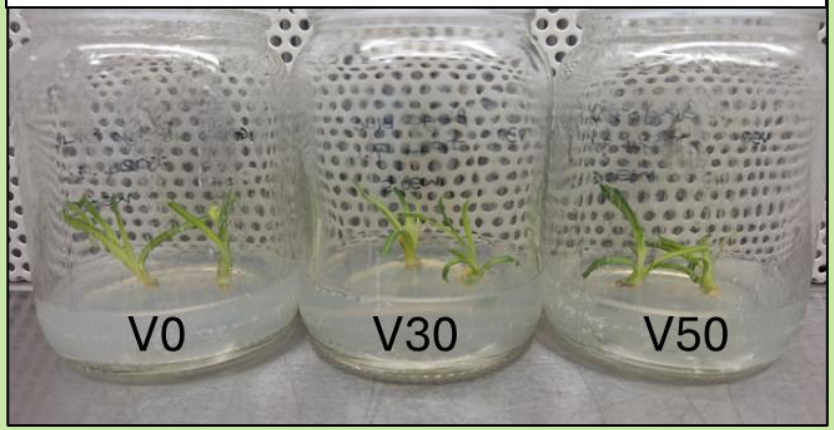
Agapanthus Chemotherapy Results after 28 days on viricide



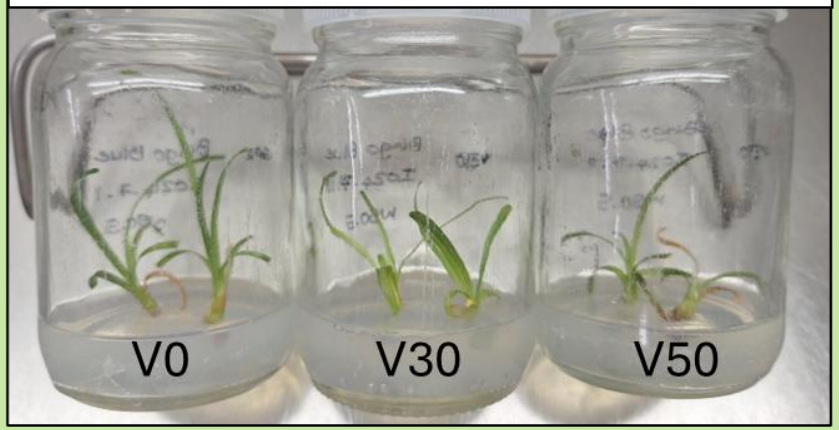
BB and BP still in good health – grown for another 12 days on viricide = Total 40 days exposure

Agapanthus Chemotherapy Results after 40 days on viricide

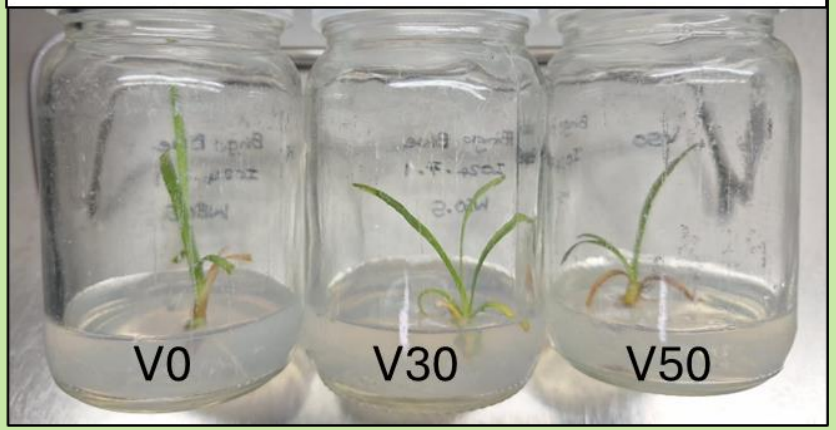
BB 0 Days



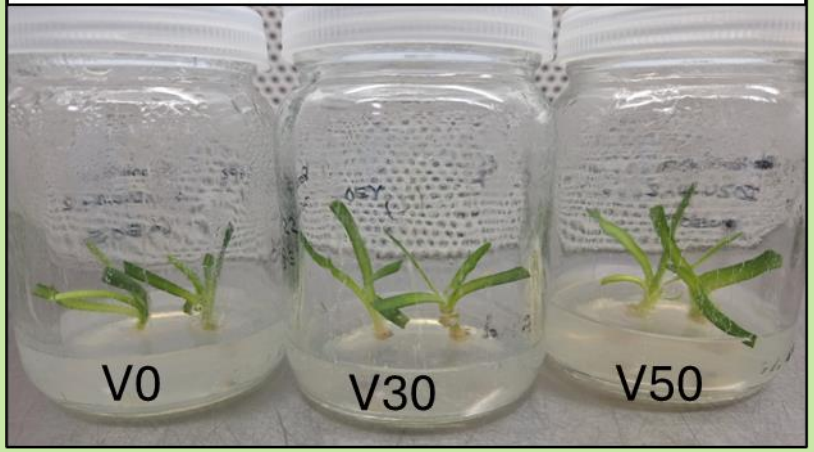
BB 28 Days



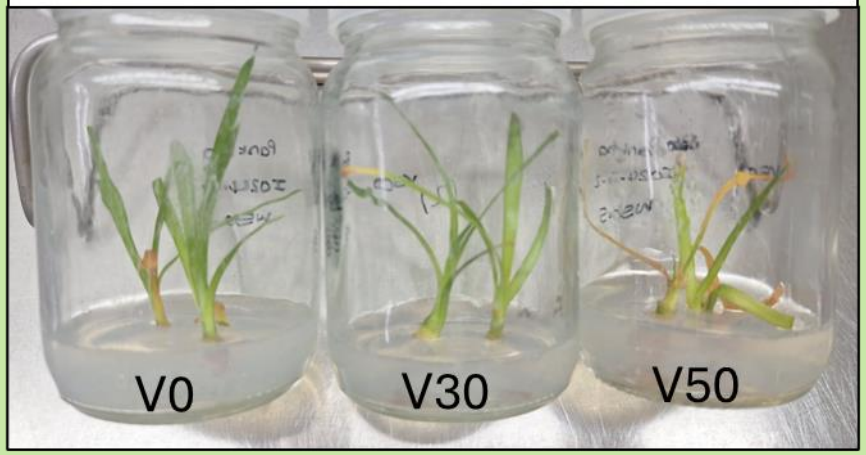
BB 40 Days



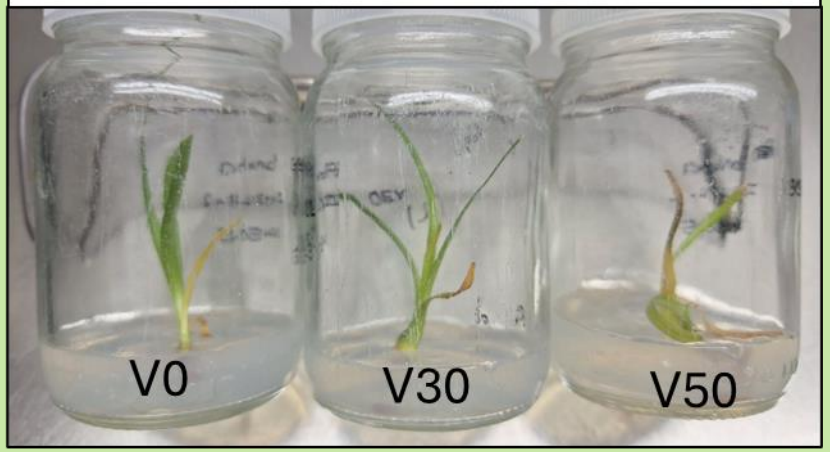
BP 0 Days



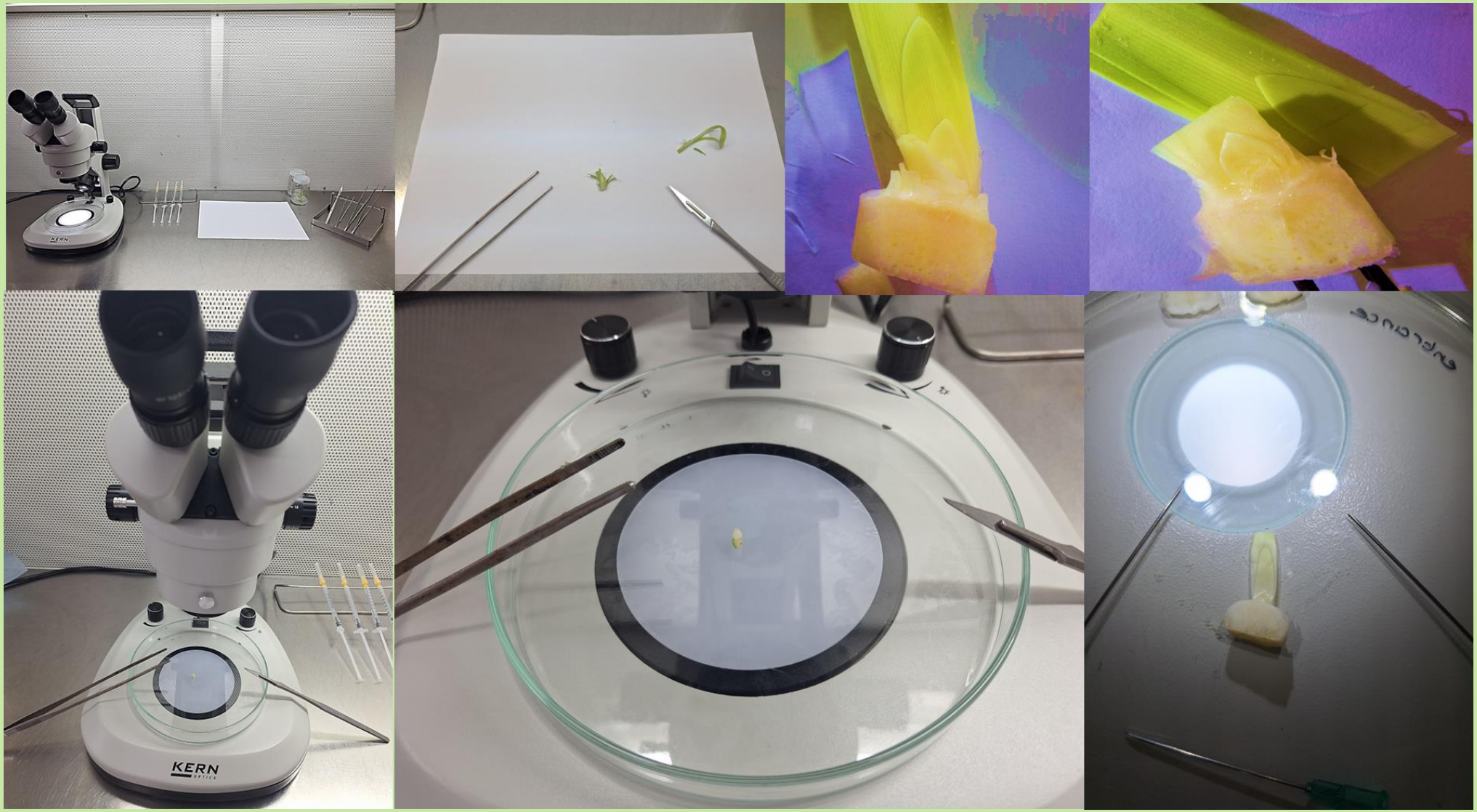
BP 28 Days



BP 40 Days

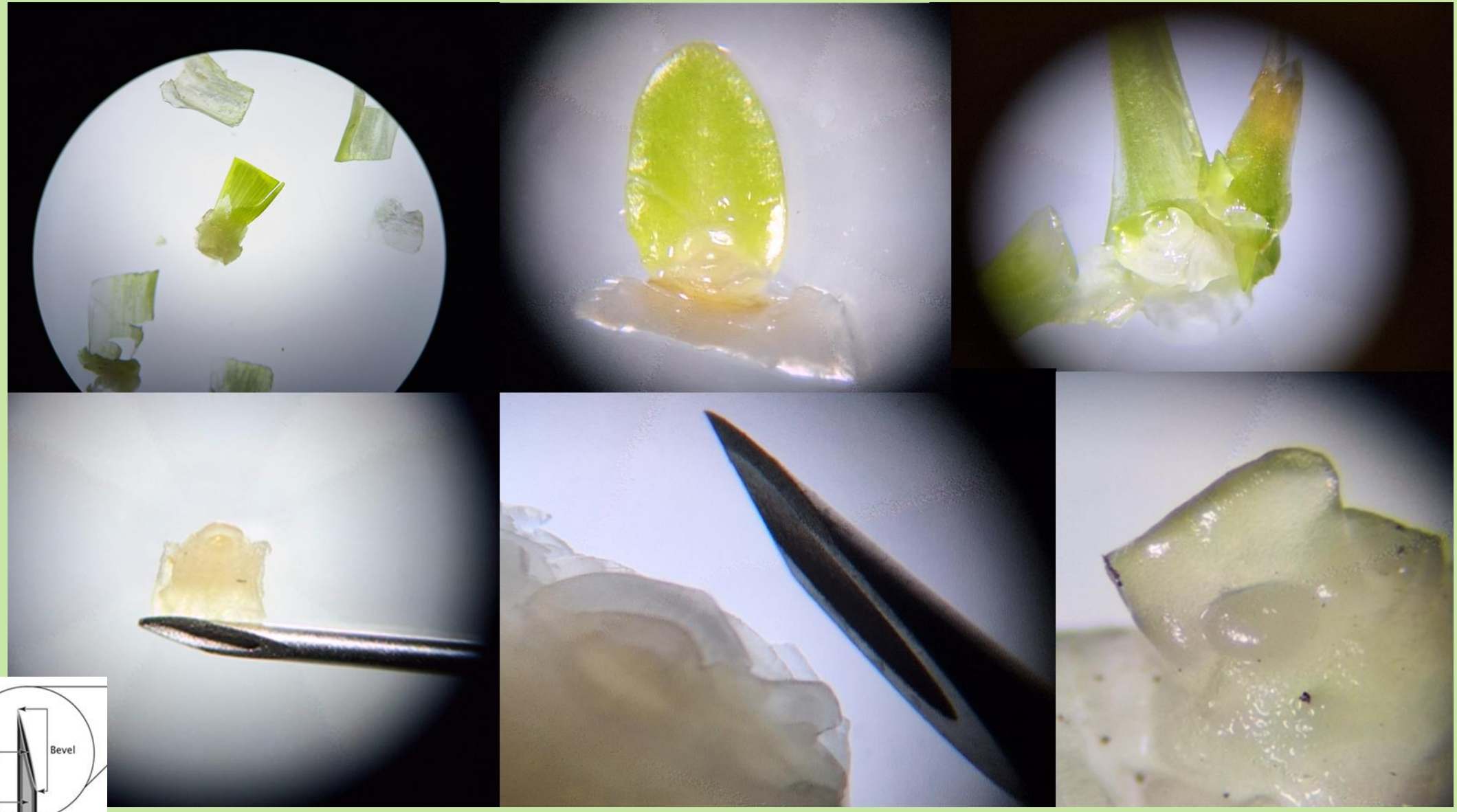


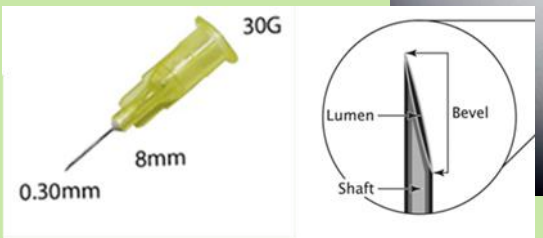
Agapanthus Meristem Excision



Agapanthus Meristem Excision

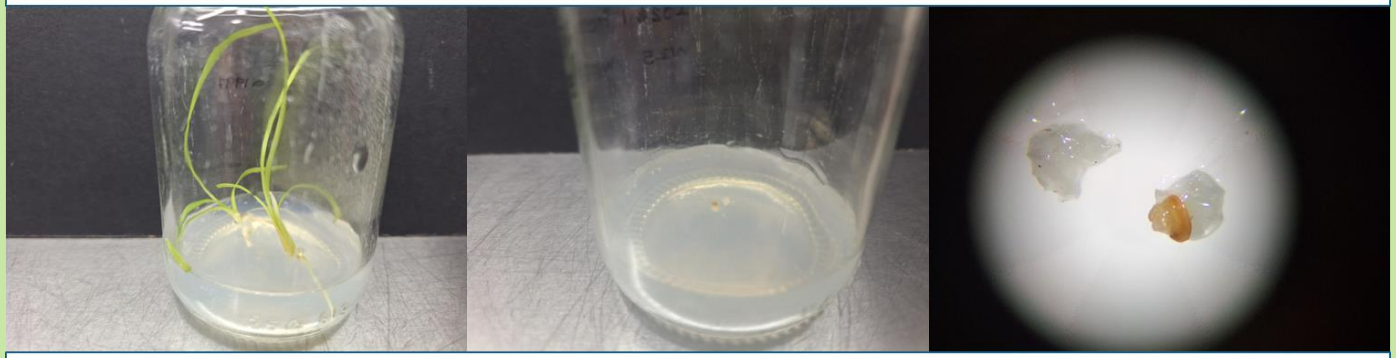
- Use 30G syringe needles = needle width 0.3mm



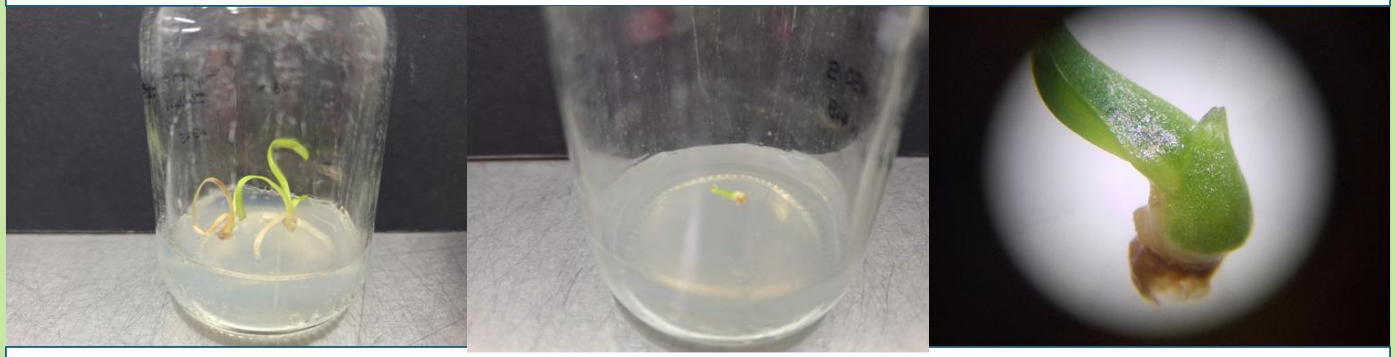


Agapanthus SN Meristem Excision

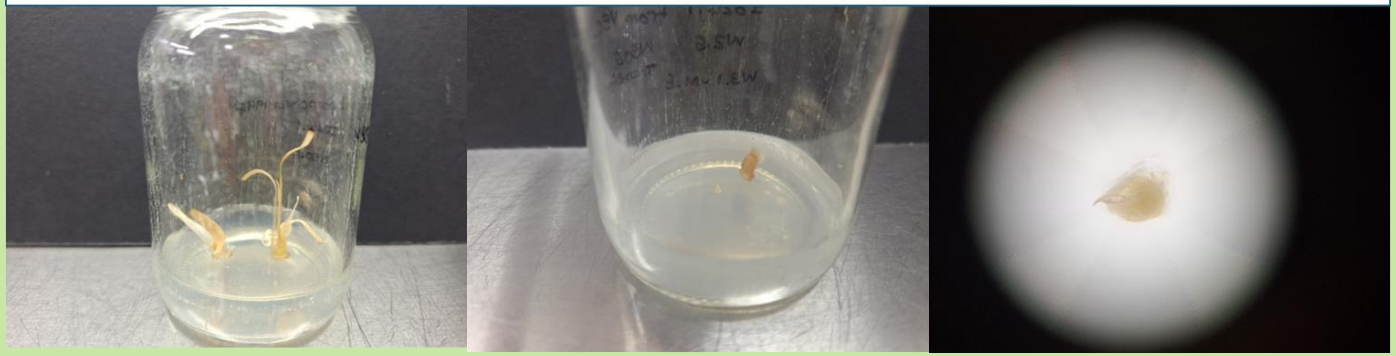
SN V0 for 28 days – Meristem grown for 4 weeks



SN V30 for 28 days – Meristem grown for 4 weeks

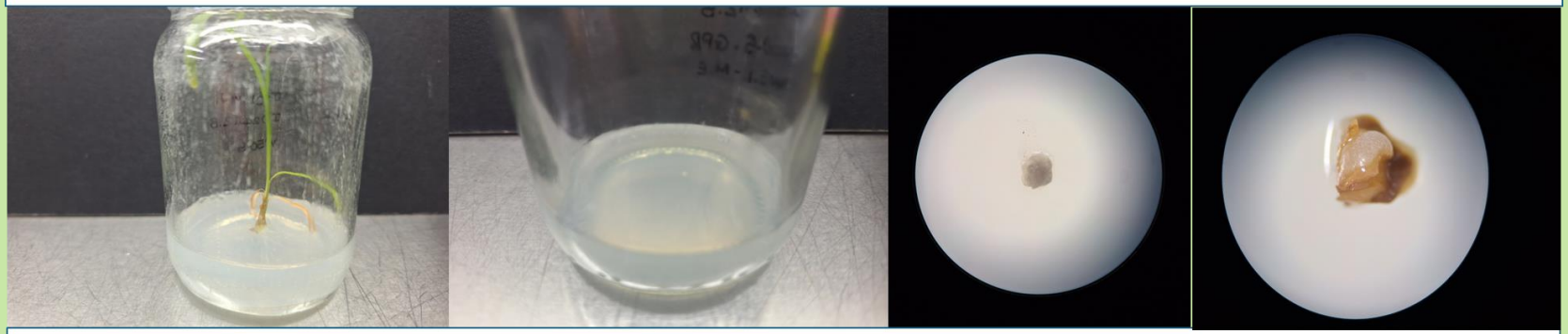


SN V50 for 28 days – Meristem grown for 4 weeks

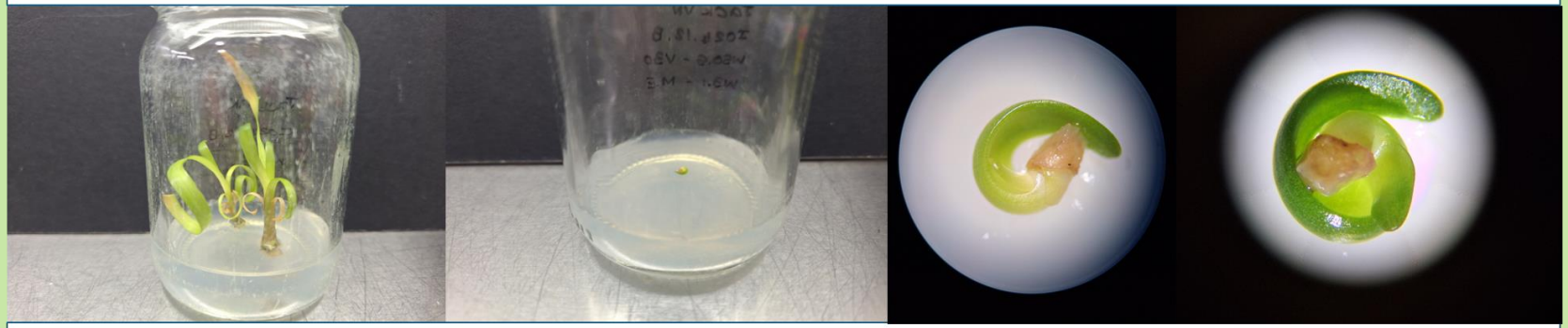


Agapanthus BJ Meristem Excision

BJ V0 for 28 days – Meristem grown for 4 weeks – 6 weeks



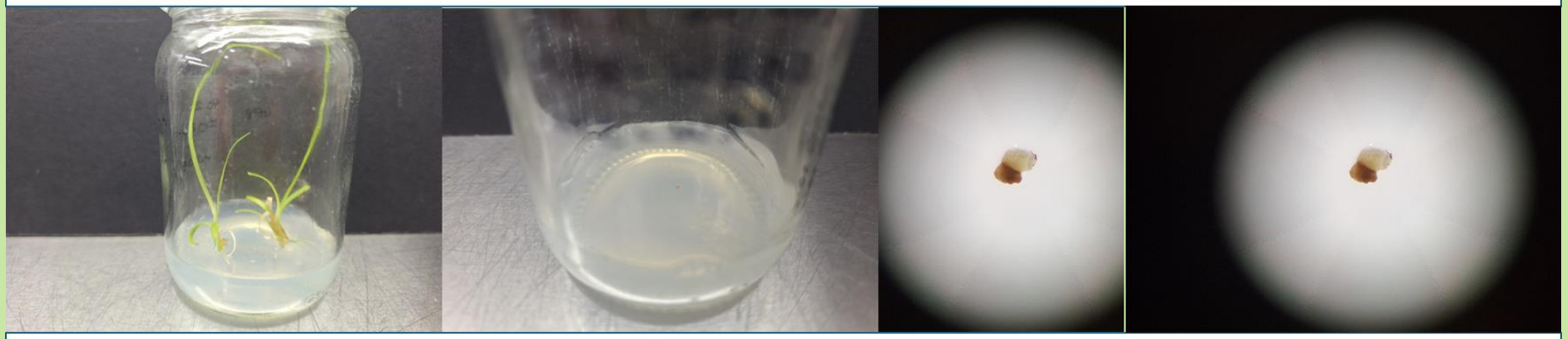
BJ V30 for 28 days – Meristem grown for 4 weeks – 6 weeks



BJ V50 for 28 days – Meristem grown for 4 weeks – 6 weeks

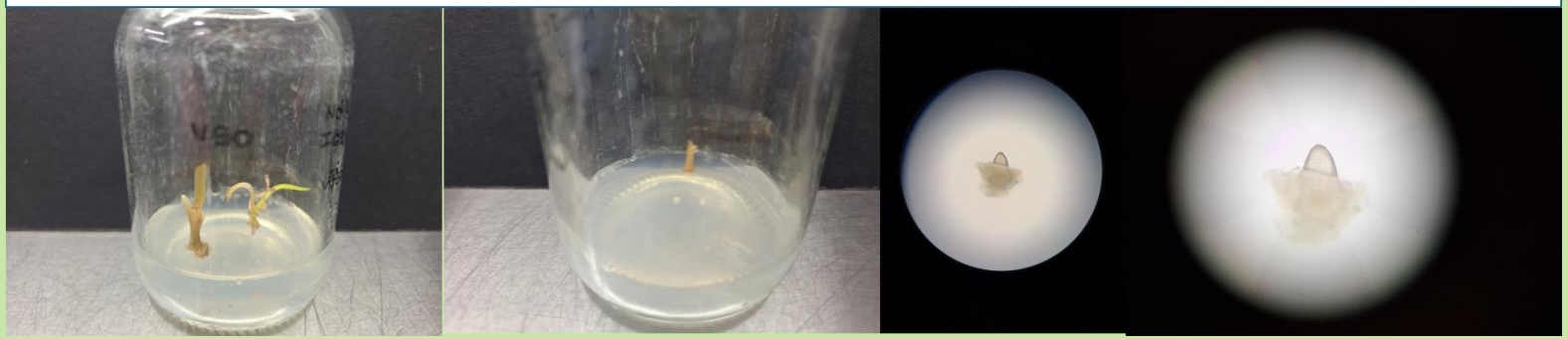
Agapanthus N2 Meristem Excision

N2 V0 for 28 days – Meristem grown for 4 weeks – 6 weeks



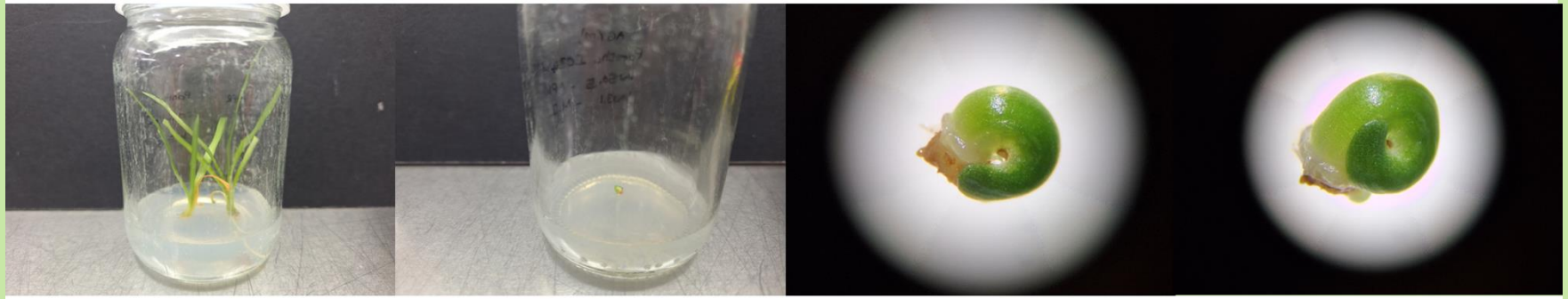
N2 V30 for 28 days – Meristem grown for 4 weeks – 6 weeks

N2 V50 for 28 days – Meristem grown for 4 weeks – 6 weeks



Agapanthus BP Meristem Excision

BP V0 for 28 days – Meristem grown for 4 weeks – 6 weeks

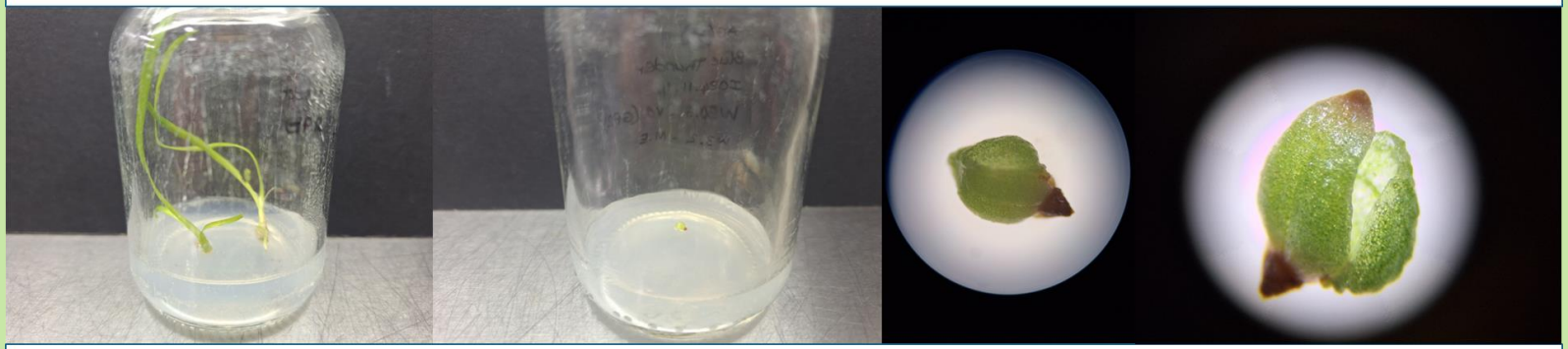


BP V30 for 28 days – Meristem grown for 4 weeks – 6 weeks

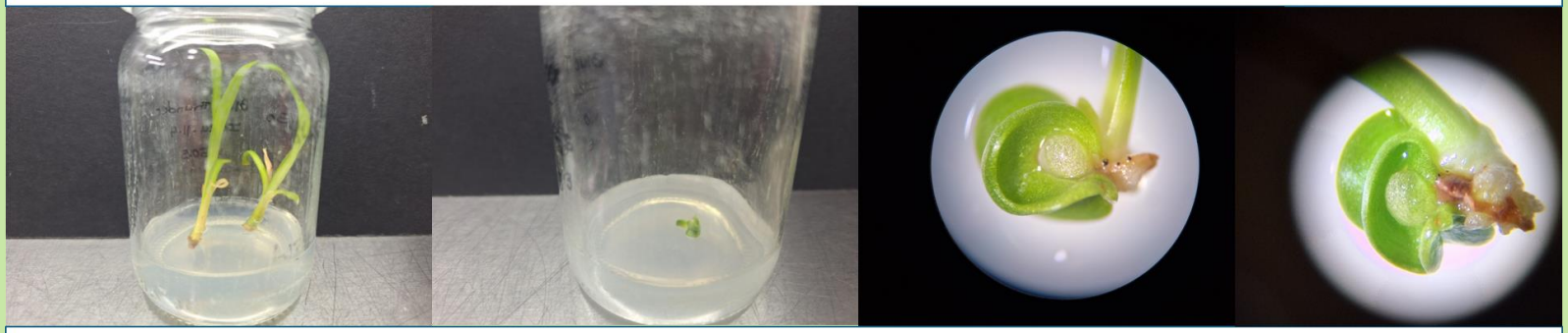
BP V50 for 28 days – Meristem grown for 4 weeks – 6weeks

Agapanthus BT Meristem Excision

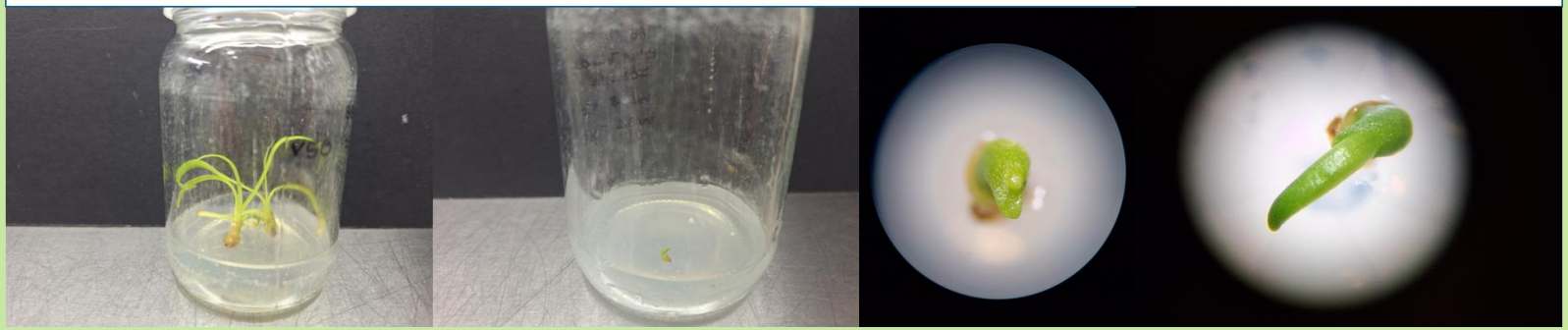
BT V0 for 28 days – Meristem grown for 4 weeks – 6 weeks



BT V30 for 28 days – Meristem grown for 4 weeks – 6 weeks

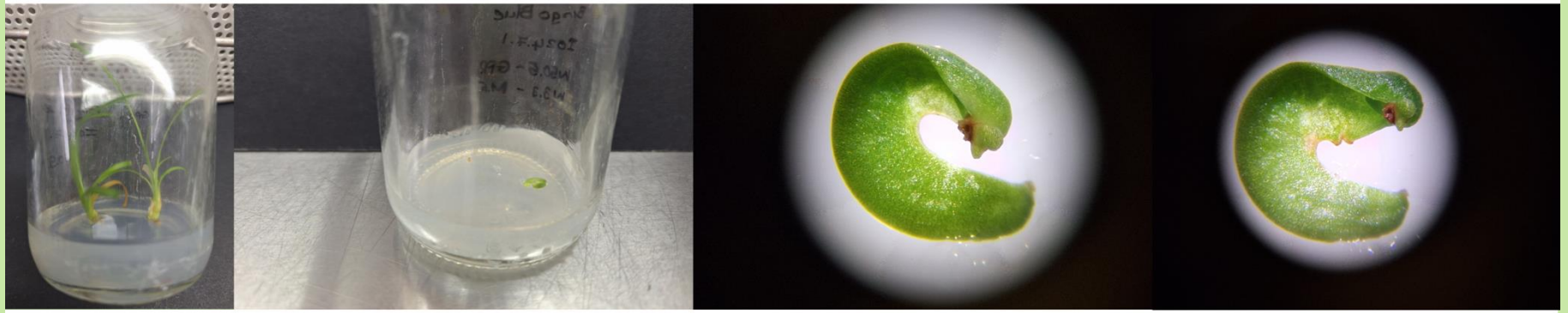


BT V50 for 28 days – Meristem grown for 4 weeks – 6 weeks

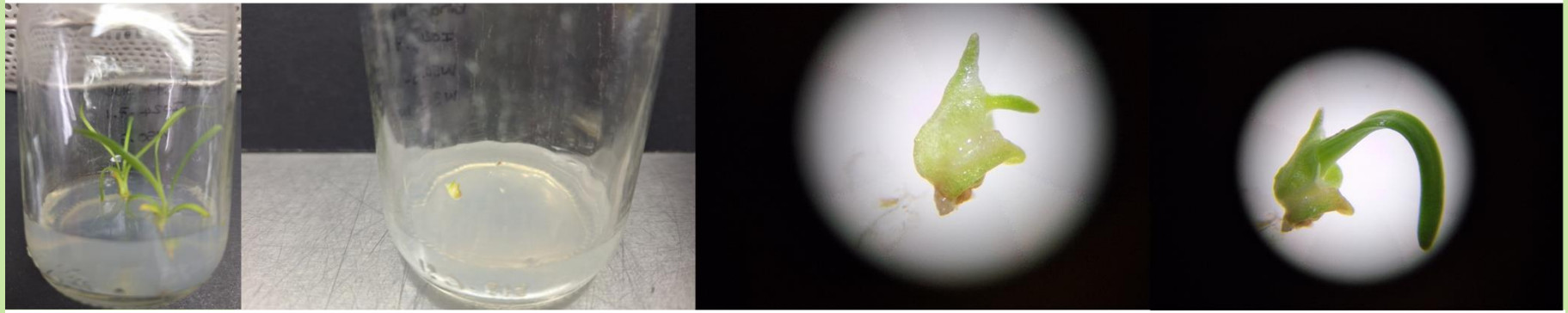


Agapanthus BB Meristem Excision

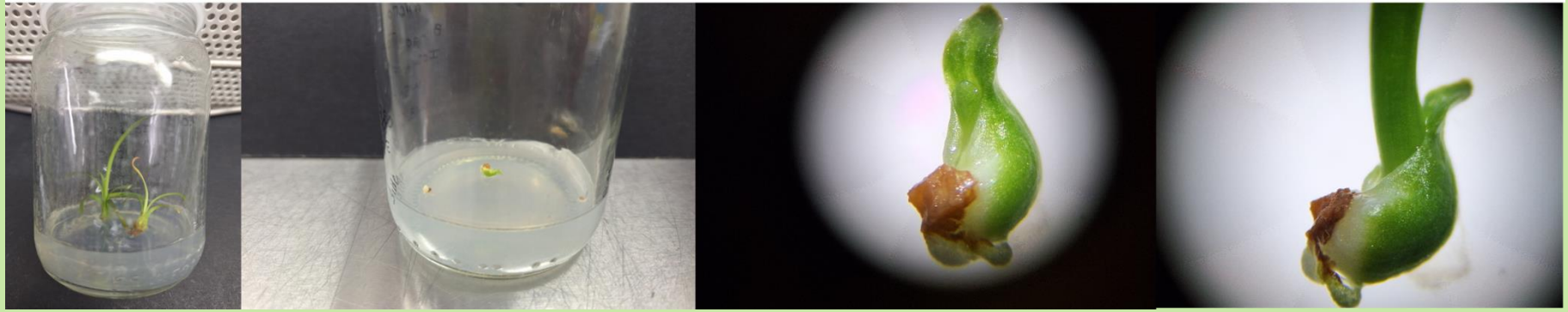
BB (*AgTV*) V0 for 28 days – Meristem grown for 4 weeks – 6 weeks



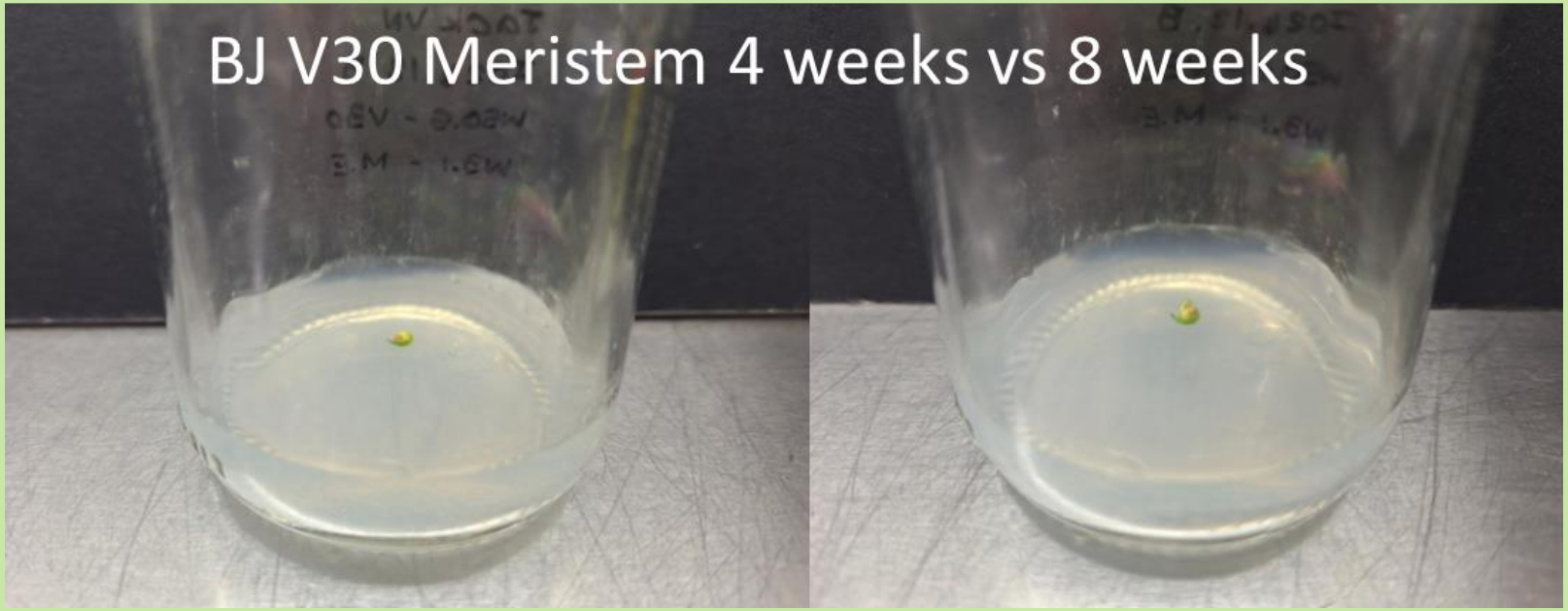
BB (*AgTV*) V30 for 28 days – Meristem grown for 4 weeks – 6 weeks

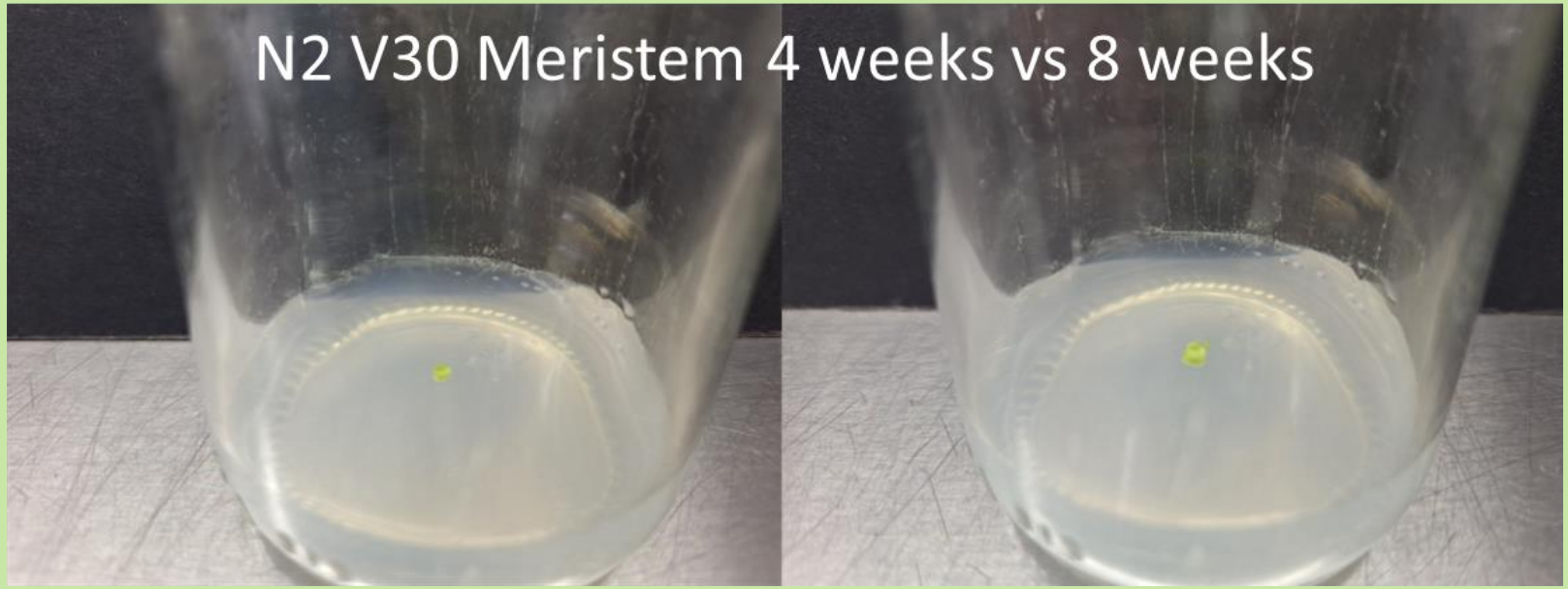


BB (*AgTV*) V50 for 28 days – Meristem grown for 4 weeks – 6 weeks



What's next?


BJ V30 Meristem 4 weeks vs 8 weeks

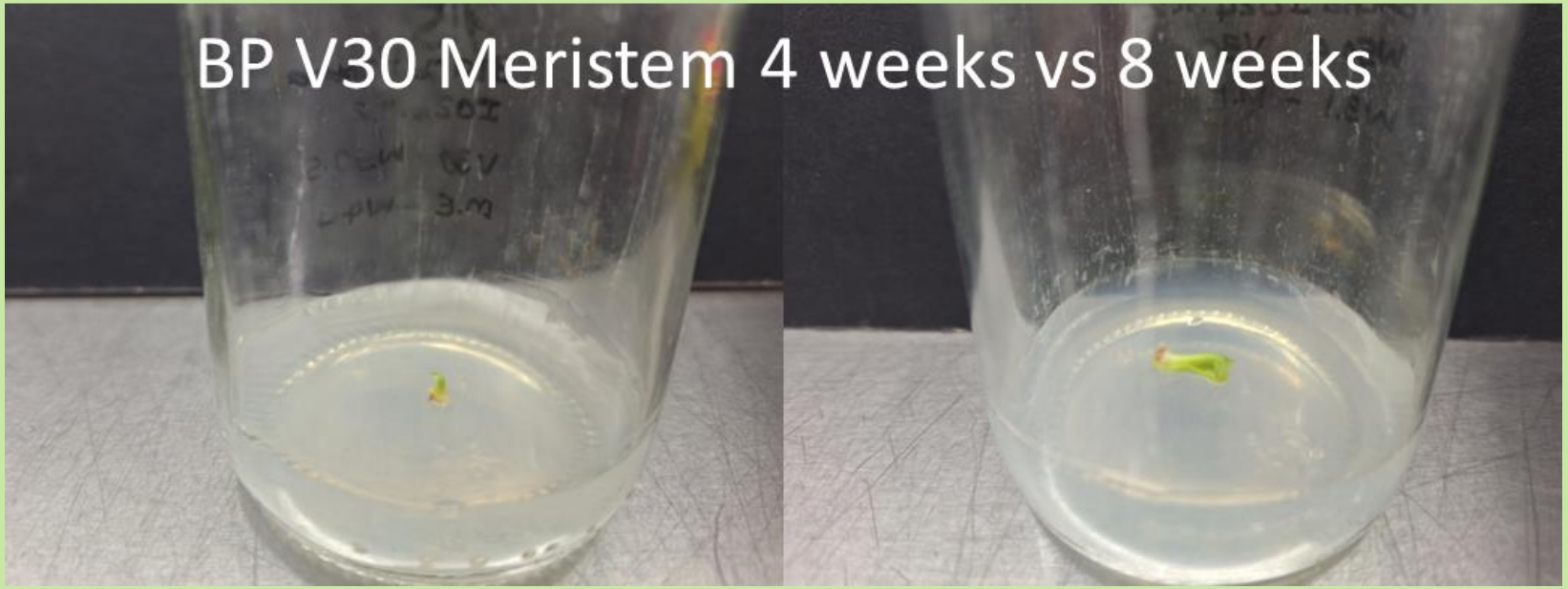

N2 V30 Meristem 4 weeks vs 8 weeks

What's next?


BT V50 Meristem 4 weeks vs 8 weeks


SN V30 Meristem 4 weeks vs 8 weeks

What's next?


BP V30 Meristem 4 weeks vs 8 weeks


BB V50 Meristem 4 weeks vs 8 weeks

New study – Kiwi fruit

More than 20 known viruses infect kiwi fruit

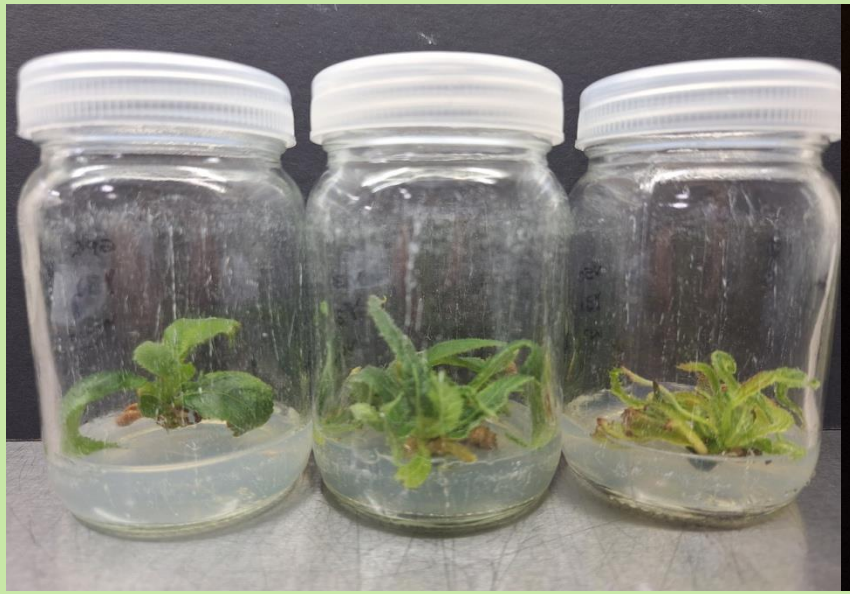
Ten kiwi varieties pooled and tested with NGS

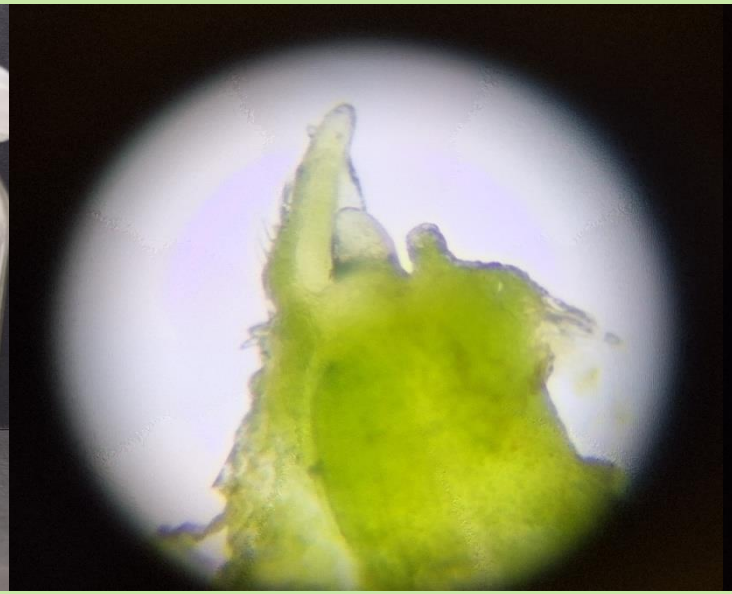
Only one virus present

Actinidia seed-borne latent virus (ASbLV)

Individual varieties tested – only two infected

Virus present	Support for finding	Comments
Actinidia seed borne latent virus (ASbLV)	99.1% of reference mapped at high stringency by reads. A single contigs of 8174bases mapping 99.8% of the reference at high stringency	The virus is transmitted efficiently via seed. ASbLV infections are typically latent — plants appear healthy with no obvious foliar or growth symptoms. The virus has been reported from New Zealand and China.






Thank you for your time and patience