# Innovations for rapid identification of pests and diseases
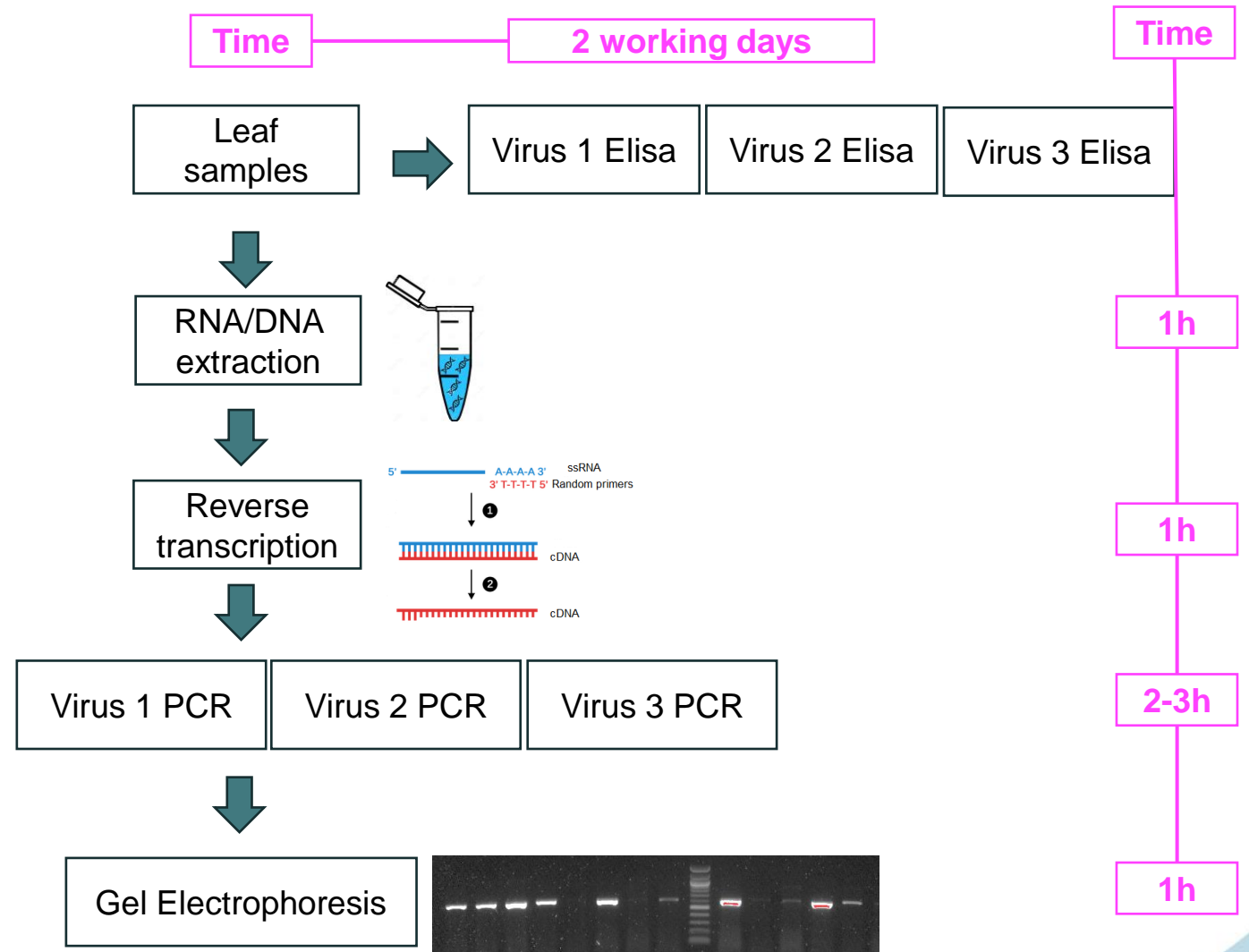
Adoption of modern quantitative PCR (qPCR) and HTS platforms as diagnostics standards

Chi Cao

Research Scientist

Grains Virology

# Current situation in Grains Diagnostic

Crops and viruses to be surveyed in Virus management project

| Crop type | Viruses tested |
|-----------|----------------|
| Cereals | YDVs (+species) |
| | WSMV |
| Canola | TuYV |
| | TuMV |
| Lupins | BYMV |
| | CMV |
| Field peas | PSbMV |
| | TuYV/PBMYV/SbDV |
| Lentils | CMV |
| | BYMV |
| | AMV |
| | TuYV/PBYMV/SbDV |
| Faba bean | BYMV |
| Chickpea | TuYV/PBMYV/BLRV/SbDV |
| | AMV |
| | CMV |

**Time** —— **2 working days** — **Time**

Leaf samples → Virus 1 Elisa | Virus 2 Elisa | Virus 3 Elisa

RNA/DNA extraction — **1h**

5' ———————— A-A-A-A 3' ssRNA
3' T-T-T-T 5' Random primers

Reverse transcription ❶ cDNA ❷ cDNA — **1h**

Virus 1 PCR | Virus 2 PCR | Virus 3 PCR — **2-3h**

Gel Electrophoresis — **1h**

# Multiplex qPCR assays for the detection of multiple viruses at the same time

New proposed qPCR assays:

- **Canola assay** for Turnip yellow Virus, Turnip Mosaic Virus and Cauliflower Mosaic Virus
- **Pulse assay** for Alfalfa mosaic virus, Bean yellow mosaic virus, and Cucumber Mosaic Virus
- **Cereal assay** for Barley yellow dwarf virus, Cereal yellow dwarf virus and WSMV
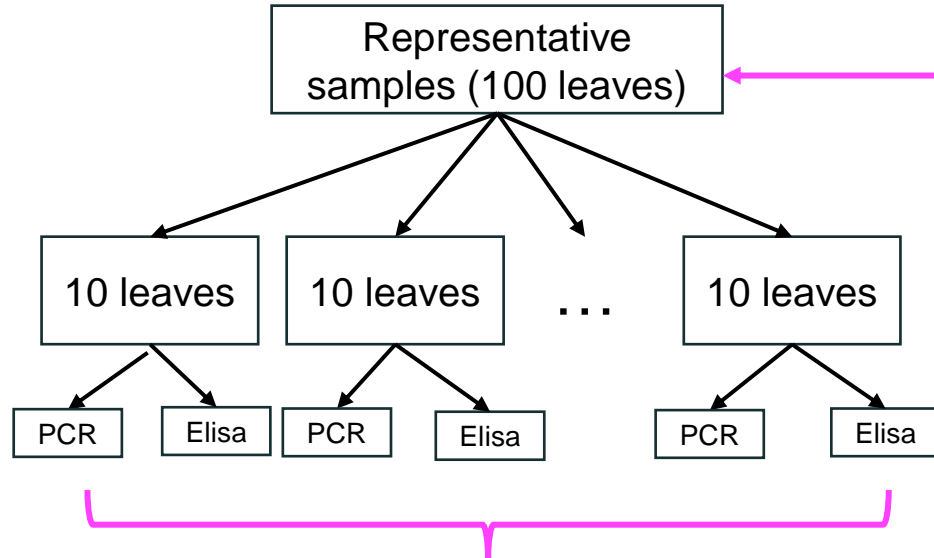- qPCR for Turnip yellows virus (TuYV) variants
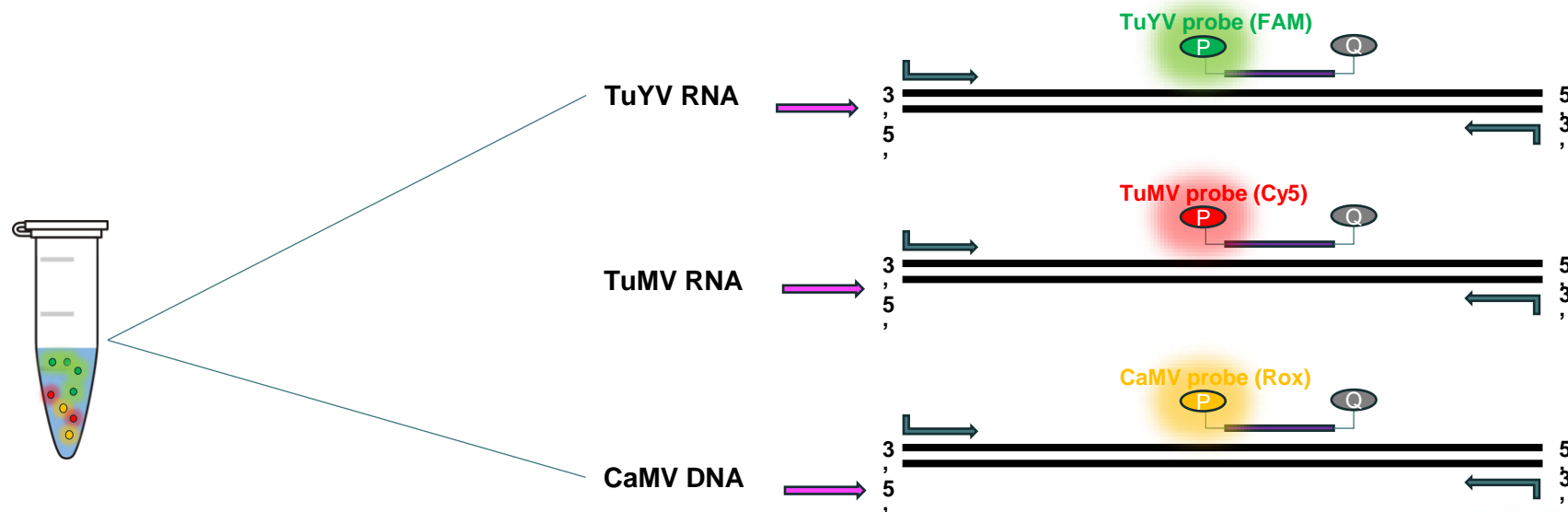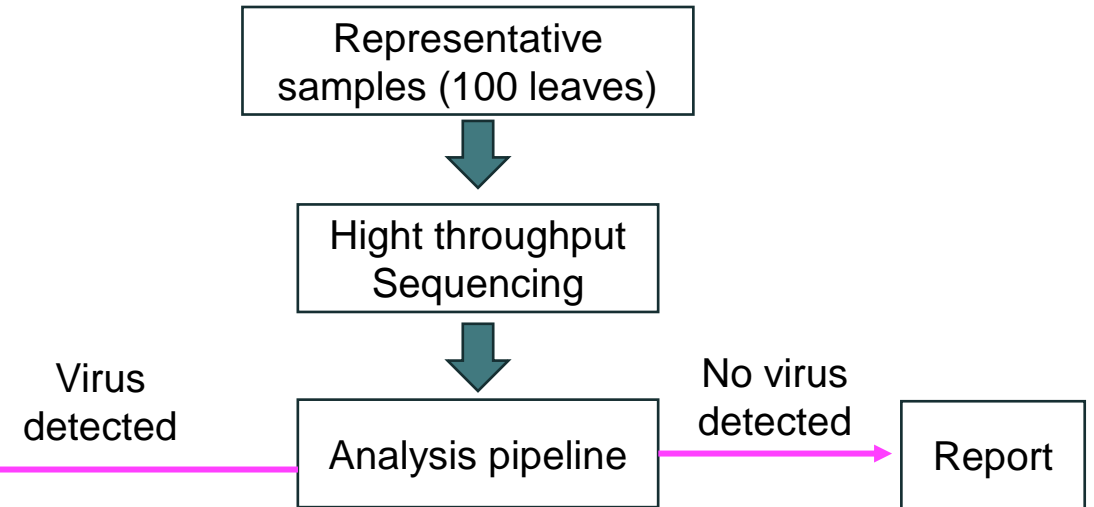
# New testing procedure
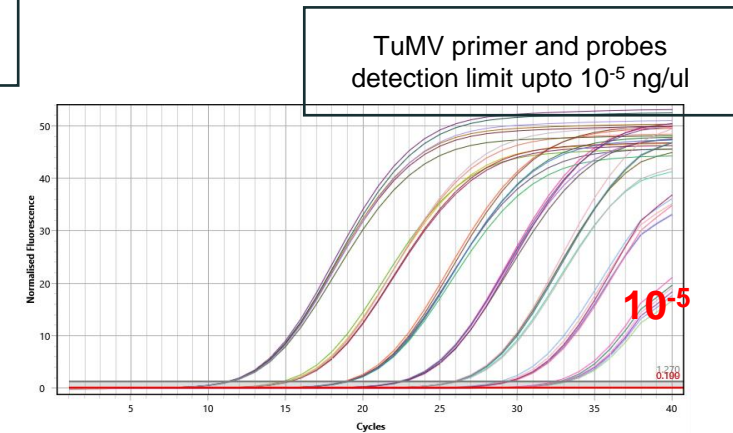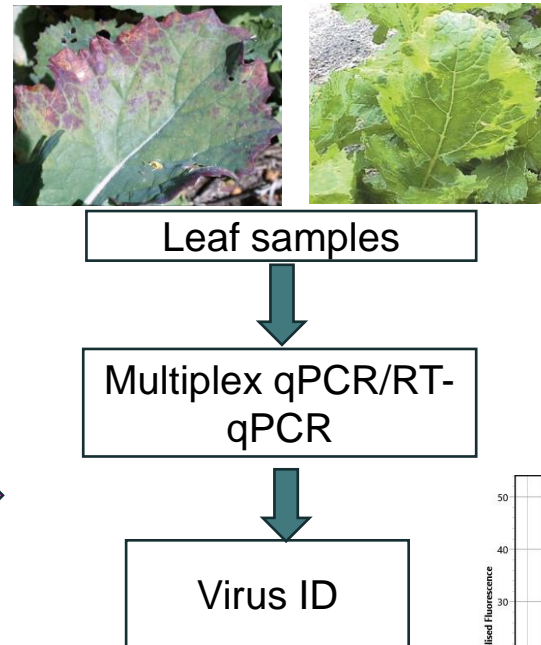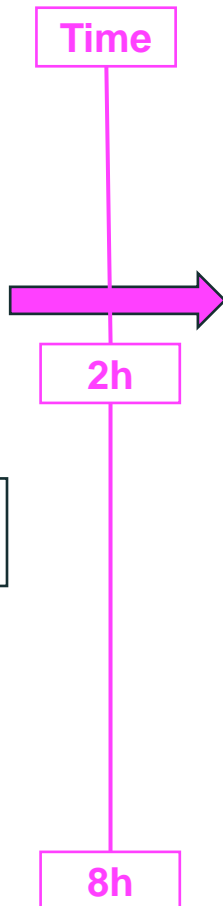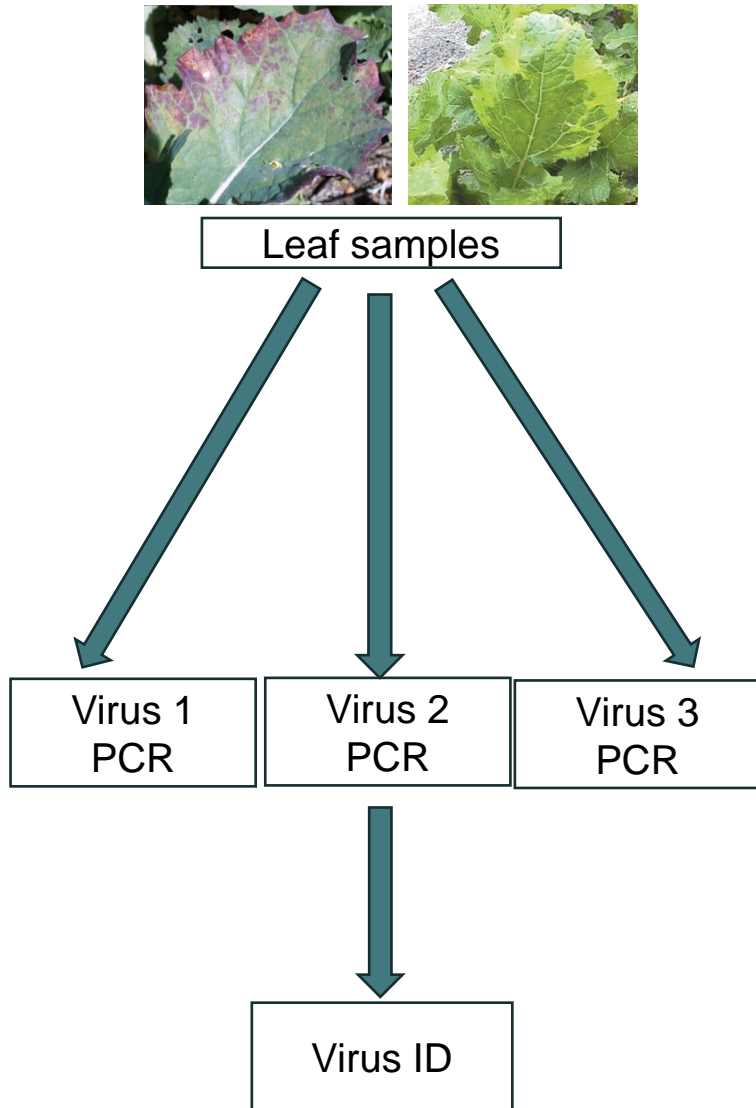


- High specificity and sensitivity
- Absolute/relative quantitative information: e.g. accurate quantification of viral load
- Efficient time, labor and materials consumption

# Using HTS in virus surveillance

**Representative samples (100 leaves)**

→ 10 leaves → PCR, Elisa

→ 10 leaves → PCR, Elisa

… → 10 leaves → PCR, Elisa

01 Pulse crop sample (100 leaves) tested with 3 to 6 common viruses
Surveillance campaign normally have in 50-60 representative samples
In many cases the crop is negative for all but one of these viruses

- Answers for YES/NO questions: Infected with a specific virus or NOT
- Information about incidence % estimation
- Time and labor and materials consuming

**Representative samples (100 leaves)**

→ Hight throughput Sequencing

→ Analysis pipeline

Virus detected →

No virus detected → Report

- Answers for YES/NO questions: Infected with a specific virus or NOT (Diagnostic purpose)

**AND …**

# Using HTS in virus surveillance

**AND ...**

- **Early warning tool:** detection of virus at early developing stage
- New genetic variants, virus strains and **resistance breaking strains** are detected
- Unexpected/new viruses, new **biosecurity threatening** viruses
- Unexpected **beneficial** viruses also can be detected
- Efficient procedure of testing:
  - Procedure efficiency
  - Cost efficient, Time efficient

# Using HTS in other surveillance

Wide-scale applicability to other organism groups e.g. fungi, bacteria, arthropod pests



Aphid trap samples → RNA/DNA extraction → Hight through put Sequencing → Analysis pipeline →
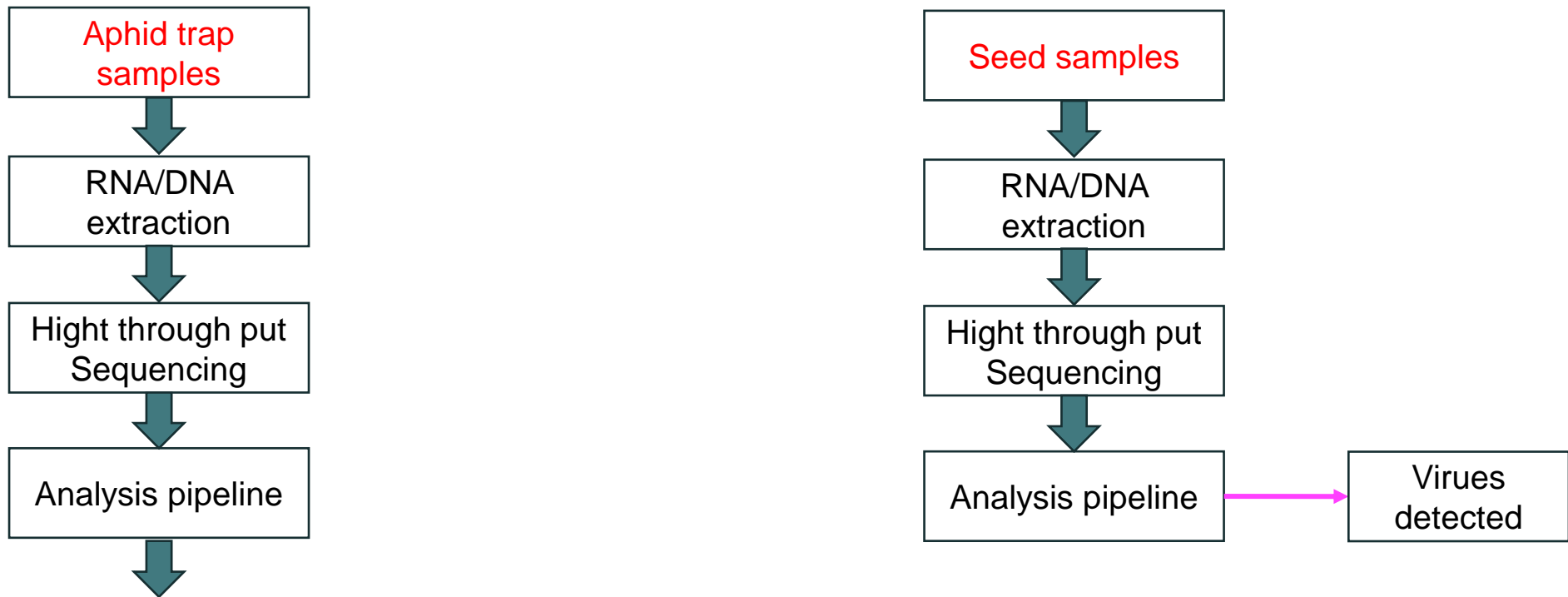
- Identification of beneficial and pest species of arthropods
- Information about symbiotic or pathogenic microorganisms inside them

Seed samples → RNA/DNA extraction → Hight through put Sequencing → Analysis pipeline → Virues detected

# Acknowledgements

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- Collaborators in Agricultural Departments interstate

# Thank you

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