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Plant Protection Department

Detection of Virus and Nematodes from Seeds

Second Lecture /3rd. stage

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2023-2024

19/10/2023

Bacterial symptoms on seeds

Bacterial panicle blight

Brown discoloration

The major seedborne nematodes are:

Bursaphelenchus cocophilus

Anguina Tritici

Aphelenchoides besseyi

Ditylenchus spp

Pratylenchus brachyurus

The common seedborne diseases caused by nematodes

Root knot nematode

Meloidogyne spp.

Potato rot nematode

Ditylenchus destructor

The common seedborne diseases caused by nematodes

Rice white tip nematode

Aphelenchoides besseyi

Seed gall nematode

Anguina tritici

Methods for detection of seedborne nematodes

1- Dry seed inspection

2- seed washing test

3- baermann funnel

4- flotation method

Dry seed inspection

The seed-gall nematode, *Anguina* spp., which replaces grains with gall-like structures,

can be detected by visual examination.

Washing seeds or galls

Diagnosis is confirmed by soaking seeds or galls in water for 1 hour and cutting them into pieces in drops of water.

Active larvae are released into the water and can be seen under a binocular microscope.

Baermann funnel

The seeds can be split longitudinally to facilitate nematode removal

- Place cutting seeds on the cotton-wool milk filter placed within sieve
- Submerge sieve with sample gently in the water of the funnel/dish
- Nematodes leave the plant tissue, pass through the cotton-wool milk filter and sink to the bottom of the funnel stem or dish,

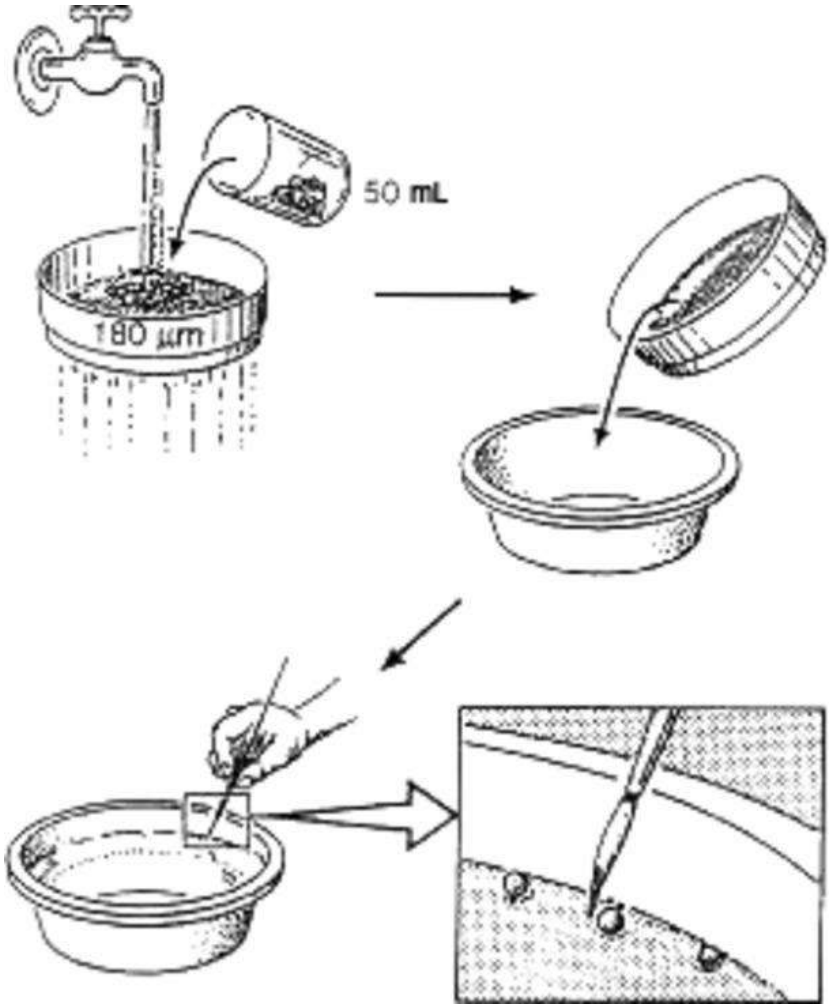
the dish is placed under microscope to detect nematodes.

Baermann funnel test

Flotation method

The seeds are placed in the salt water of 8% NaCl and stirred continuously for 10 or 15 min before keeping it for 24 h without disturbing it.

After this period, the solution is allowed to pass through the funnel containing filter paper for complete drain; then the sediment on the paper is examined for nematode cyst, galls, or egg masses of nematodes



Testing Methods for Detection of seed borne viruses

1- Visual examination

2- Indicator plant test

3- Seedling symptoms test

4- Staining test

5- Serological test

6- PCR test

Visual examination

seed morphology like shape, seed coats, color, size, and weight indicates the presence of virus.

The virus-infected seeds are usually **shrunken** in shape, having **shriveled** and **discolored seed coat**, **smaller in size**, and **light weighed**.

Sometimes, the infected seeds are cracked and may have necrotic spots on their surface.

Symptomatology

Bean yellow mosaic virus

Bean pod mottle virus

Symptomatology

Pea seed borne mosaic virus

Bean pod mottle virus

Indicator plants

Indicator plants are the plants which produce characteristic symptoms having low incubation period after mechanical inoculation.

Some of indicator plants are:

Petunia violacea, Chenopodium quinoa, C. murale, C. amaranticolor, *Phaseolus vulgaris*, and *Vigna sinensis*

mosaics, vein clearing, chlorosis, stunting, necrosis are the characteristic symptoms on the inoculated indicator seedlings.

Indicator Plant Test

Abnormal seeds are soaked in water and then triturated.

The slurries produced are then applied to indicator plants.

The slurries containing virus inoculated onto the indicator plants followed by immediate rinsing of leaves with water.

The inoculated seedlings and newly appeared young leaves are observed for symptom expression.

Grow-out Test

Grow-out test is done to check the percent seed transmission of viruses in the infected seeds based on the visual characteristic symptoms produced by the particular host-virus interaction.

Grow-out Test

Infected seeds are sown in sterile soil under controlled glasshouse environment either in Petri plates or trays or in moist towel papers.

The newly emerged leaves of the seedlings are monitored for characteristic and prominent symptoms that appeared according to a particular virus-plant association.

detection the presence of LMV in lettuce by grow-out test

after 2–3 weeks when seedlings displayed typical mosaic symptoms.

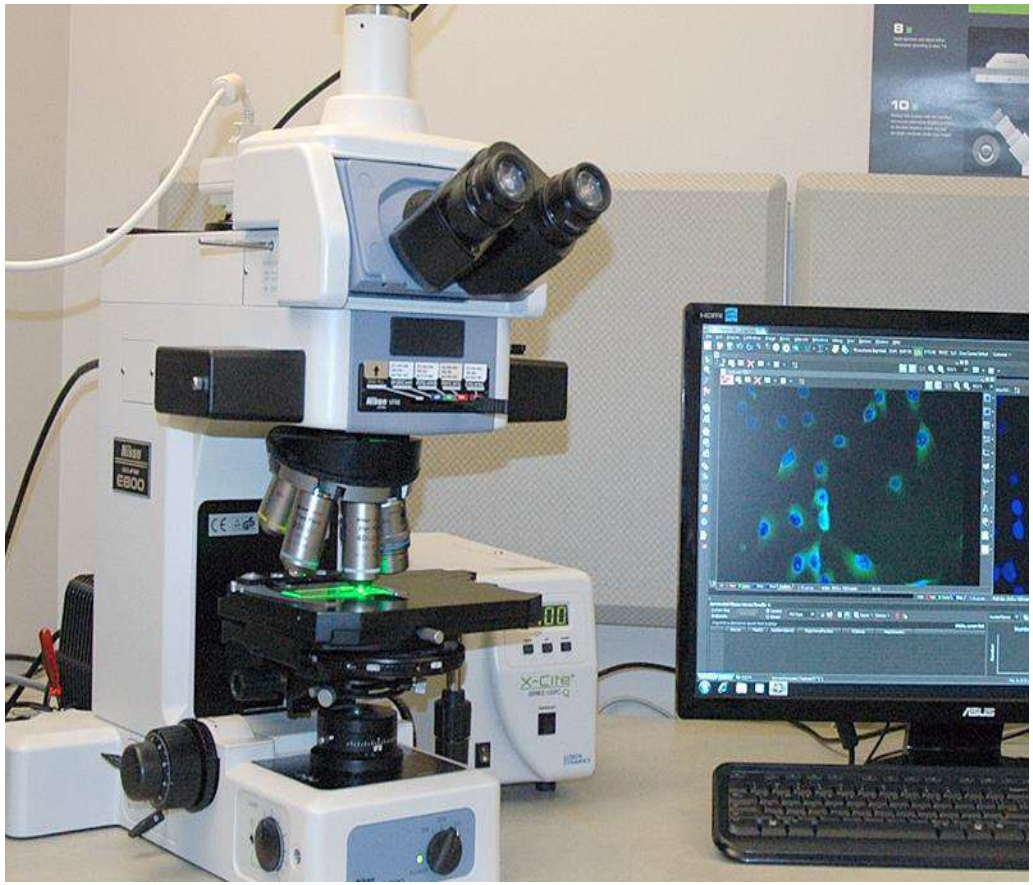
Detection of Alfalfa mosaic virus from seedlings by grow-out test

Staining test

A number of stains such as acridine orange, giemsa, phloxin, trypan blue, rose bengal etc. can detect virus infections in plant tissues.

For example : Cowpea seeds are soaked in water for 2 days, then sections are cut from the germinating embryos, stained with acridine orange, and viewed under a fluorescent microscope.

The virus is detected by aggregates of red fluorescing material in infected tissues.



Stains used to detect viruses

Acridine orange

Trypan blue

Serological test

Serological tests are based on the reaction between an antiserum, a blood serum containing specific antibodies produced by injecting laboratory animals with a pure virus preparation, and an antigen-virus protein.

Common serological tests are as follows:

1- ELISA test

2- Microprecipitin Test

3- Gel-Diffusion Tests

4- Immunofluorescence Microscopy Test

5- Agglutination test

Antibody (immunoglobulin): are protective proteins produced by immune system in response to a specific antigen and capable of reacting with that antigen

Antigen (immunogen): any foreign substance which, when introduced will evok a specific immune response

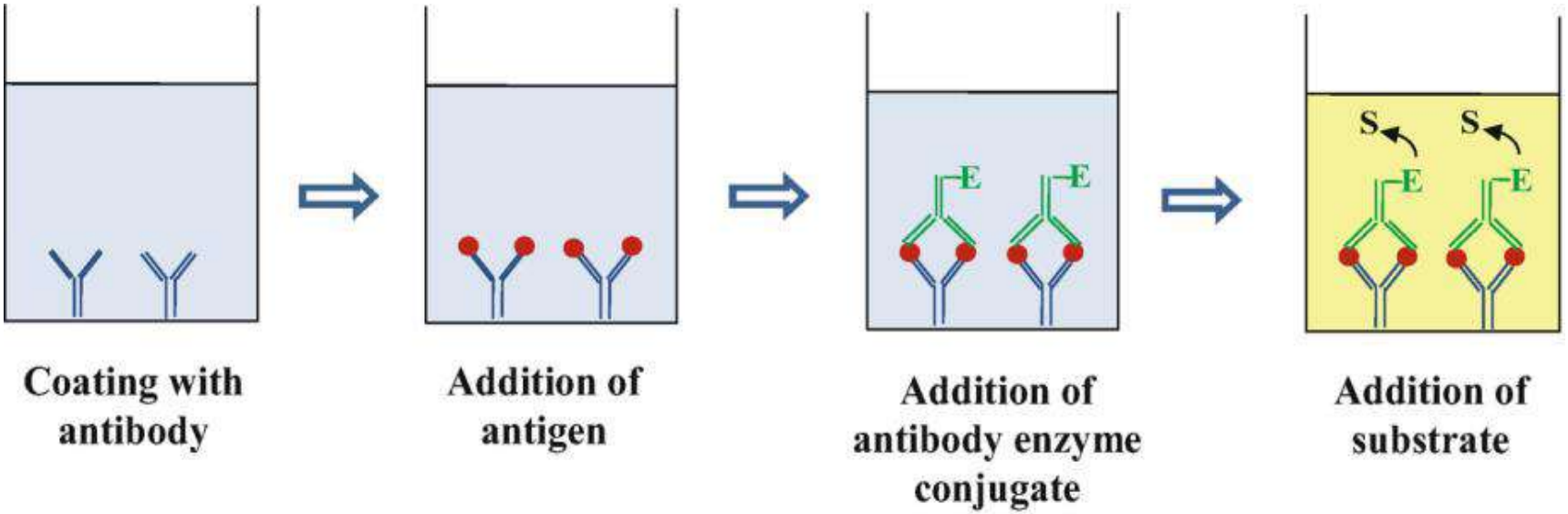
Enzyme-Linked Immunosorbent Assay

ELISA is a serological method for the identification of viruses based on antibodies and color change in the assay.

In this method, antigens from the viruses are made to specifically bind with antibodies conjugated to an enzyme.

The detection can be visualized based on color changes resulting from the interaction between the substrate and the immobilized enzyme.

The microtiter wells are first coated with the antibody-containing immunoglobulin fraction of antiserum to the virus to be assayed. After washing the wells, the virus sample is added, and after one more washing, primary antibodies labelled with an enzyme (conjugate) are added. Following another washing, enzyme substrate is added, yielding a coloured product.



Polymerase Chain Reaction (PCR) Test

PCR is an *in vitro* method of nucleic acid synthesis by which a particular segment of DNA can be amplified which detect organism specific DNA / RNA sequence.

This technique is used in the development of molecular diagnostics for the detection of seed borne pathogen. Potential benefits (e.g. rapid, same-day analysis, specific and sensitive tests)

two oligonucleotide primers were designed to detect these pathogens in DNA extracts from seed macerates.

PCR technique steps

DNA extraction

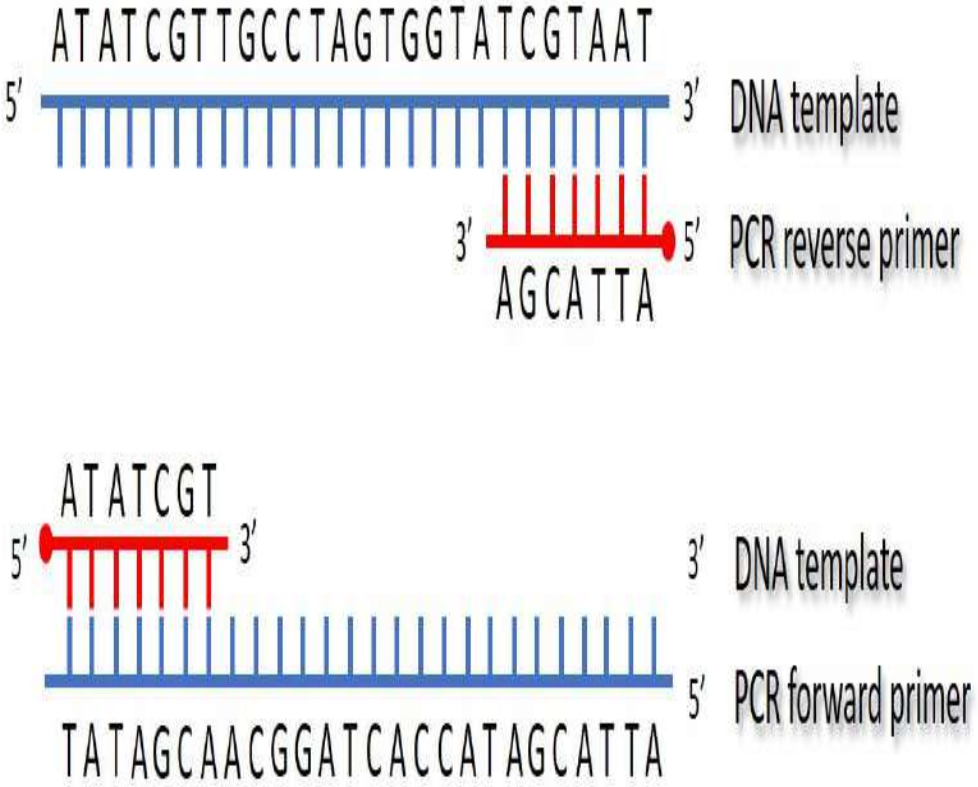
Primer design

PCR amplification

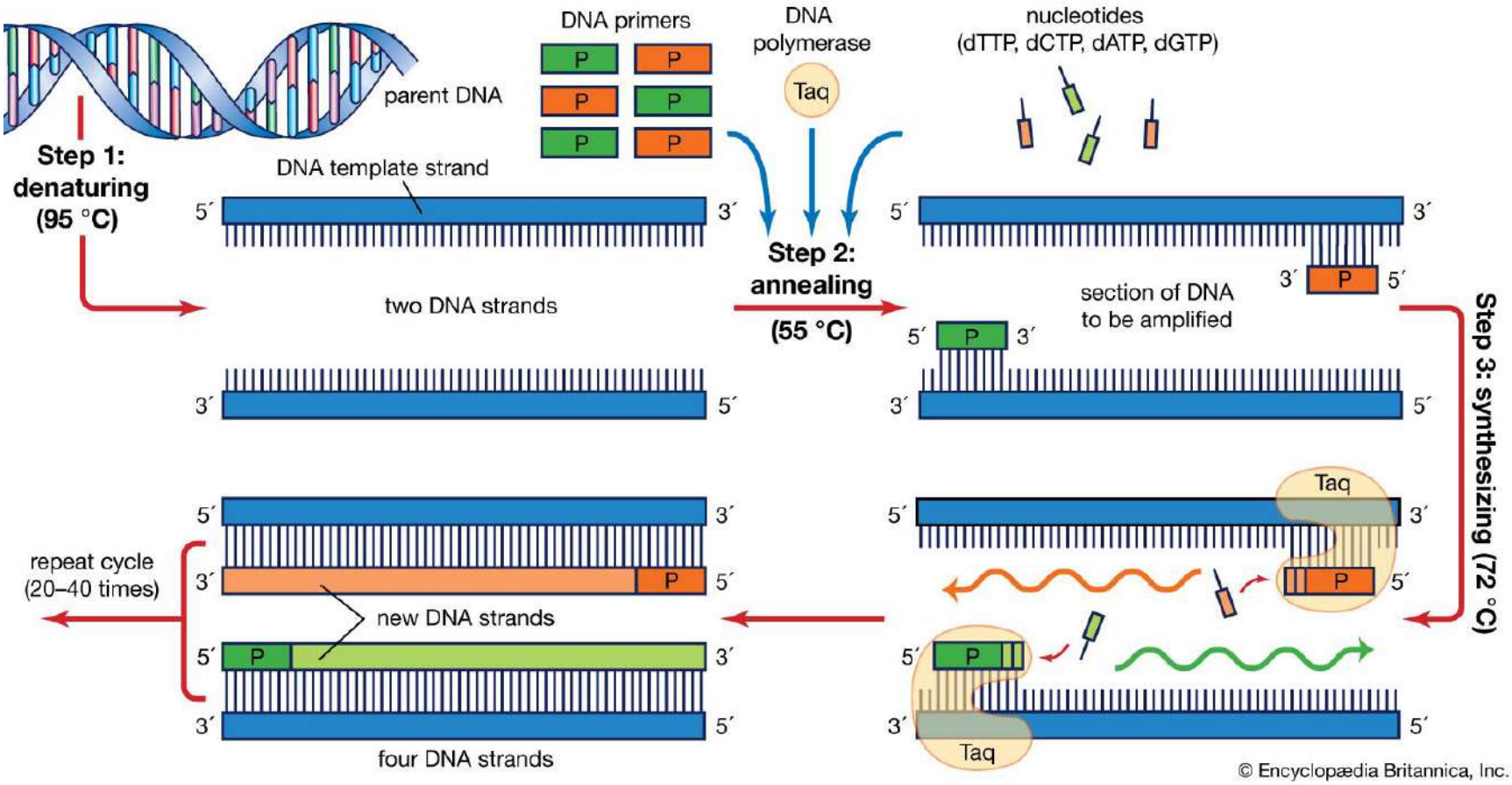
gel electrophoreses

primer

A primer: is a short, single-stranded DNA sequence used in the PCR technique. In the PCR method, a pair of primer is used to hybridize with the sample DNA and define the region of the DNA that will be amplified. Primers are also referred to as oligonucleotides.



PCR amplification



Gel electrophoreses