DETECTION OF SEEDBORNE PATHOGENS

Methods of detecting seed infection

1. Inspection of dry seeds – detects the presence of fruiting structures of fungi and the effects of fungi on the physical appearance of seeds
2. Blotter methods – this is a simple and inexpensive way to detect seedborne fungi that respond to sporulation
3. Agar plate methods - Detects and identifies seedborne fungi through colony characteristics which they exhibit when grown on nutrient agar
4. Seed washing test - reveals identifiable spores or mycelia adhering to or growing on the seed surface
5. Growing-on test - Detects seedborne fungal, viral, and bacterial pathogens which are readily transmittable.

Requirements

- Untreated and unsorted seed samples purchased from local market (maize, bean, sorghum, millet, cowpea)
- A simple purity box, with a smooth top surface - use white manila paper in absence of purity box
- Stereomicroscope (magnification at least up to x 50)
- Compound microscope (magnification up to x 400)
- Magnifying lenses
- Balance (up to 3 decimals)
- Disposable plastic Petri dishes
- Glass slides and cover slips
- Sterile distilled water
- Forceps, mounting needles, scalpel blades with handles
- Microscope lens cleaning paper
- Felt pens, paper sticker labels
- Filter papers (9 cm diameter) with high holding capacity
- Trays (30 x 60 cm) for holding Petri dishes
- Beakers
- Alcohol lamps

Inspection of dry seeds

The method provides quick information on insect and mechanical damage to the seeds. The examination of dry seeds is done during the purity test.

1. Select a random sample of 2500 seeds from a well-mixed sample from unsorted seed from local market
2. Place the seed sample on purity board (use white Manila paper in absence of lighted purity board).
3. Using a seed pushing wedge (or a knife, forceps or scalpel blade), separate the seeds into the following groups based on physical abnormalities (may use magnifying lens):
   - Shriveled seeds,
   - Reduction or increase in seed size ,
   - Discoloration or spots in the seed coat

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- Rotten seeds
- Insect damaged seeds

Count and weigh the seeds in each component group and express as percentage.

4. Using magnifying lens and stereomicroscope observe the fungal structures on rotten, discoursed and shriveled seed (the fungal structures may be mycelia, spores, acervuli, pycnidia, pericthecia, sclerotia on the seed surface or submerged in the seed coat; sclerotia may be loosely mixed with seeds).

Blotter method

1. Select a random sample of 400 seeds from a sample; surface sterilize the seeds in 2.5% sodium hypochlorite, rinse the seeds in sterile distilled water and blot dry on sterile paper towel.
2. Place three 9.0 cm filter papers in each Petri plate and soak with sterile distilled water. Drain away excess water.
3. Aseptically place 10 seeds, evenly spaced, on the surface of the filter paper in each plate. (use 5 seeds for large seeded species – maize/bean/cowpeas; 10 seeds for small seeded species - sorghum/millet).
4. Incubate for 3 days at 25^{0}C in the dark. Care should be taken while handling the dishes in the tray and transferring them to the incubation room so that the plated seeds are not displaced from their original position.
5. Transfer the plates to a freezer and maintain at –20^{0}C for 24 hours. Freezing prevents germination of the seeds.
6. After freezing, incubate for 6 days at 25^{0}C with alternating 12 hr periods of darkness and near U-V (NUV) light. Plates should be approx. 25 cm below the lights and should not be stacked. Light induces sporulation which helps to identify the fungi.
7. After incubation, bring the Petri dishes to the examination area. Examine the seeds under a stereoscopic microscope at x30 for fungal growth and up to x80 magnification for identification of spores and spore-bearing structures.
8. Record the number of infected seeds in each plate.
9. Make a slide preparations of the fruiting structures of the fungi and observe under a compound microscope

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> Seed treatments may affect the performance of this test. It should only be performed on untreated seed.

Agar Plate Method

In the agar plate method more than one type of fungal colonies are produced. The number can be 3, 4, 5 or even higher depending on the level of infection present in the seeds.

1. Prepare plates of potato dextrose agar (PDA) medium and allow to cool to 45-50^{0}C in water bath (*amend the PDA media with 20ppm streptomycin sulphate to reduce growth of bacteria – the antibiotic solution is added to media after autoclaving and cooling to 45-50^{0}C*).

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2. Select a random sample of 400 seeds from a sample; surface sterilize the seeds in 2.5% sodium hypochlorite for 3-5 minutes, rinse the seeds in sterile distilled water and blot dry on sterile paper towel.
3. Dispense the PDA medium into 9 cm plastic Peteri dishes under sterile conditions.
4. Using sterile forceps, plate 5 to 10 seeds on the surface of non-solidified agar medium (5 seed per plate for large seeded varieties – maize/bean/cowpea or 10 seeds per plate for small seeded species – sorghum/millet).
5. Incubate for 3 days at 25ºC in the dark. Transfer the plates to a freezer and maintain at -20ºC for 24 hours. Freezing prevents germination of the seeds. After freezing, incubate for 6 days at 25ºC with alternating 12 hr periods of darkness and near U-V (NUV) light. Plates should be approx. 25 cm below the lights and should not be stacked. Light induces sporulation which helps to identify the fungi.
6. Examine the fungi growing out from seeds visually and under stereomicroscope to observe colony characters and morphology of sporulating structures. Make slides of the fungal structures and observe under compound microscope. Identify the most frequently occurring fungal colonies present in all Petri dishes, then the second most frequent, then the third most frequent, and so on.
7. Record the counts of the investigated fungi from each dish

Growing-on test (Seedling Symptom Test)

The growing-on test is based on the fact that some of the seed-pathogens are capable of attacking seeds, making them ungerminable resulting in rooting of seeds, and in producing symptoms on young seedlings or even killing the affected seedlings. These effects can be seen if seeds are sown on suitable substrate and seedling grown under environmental conditions which support expression of such effects.

For this test use the germination test materials –

1. Between paper towel
2. In rolled paper towel
3. On sand

For each seed lot tested for germination using each of the above methods, count the number of seeds (express as percentage) showing infection as observed as:

- Colonization of seeds by heavy growth of fungi resulting in loss of germination (seed rotting).
- Symptoms in roots (discoloration, rotting)
- Symptoms in cotyledons, coleoptile, hypocotyls and leaves
- Death of seedlings

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