Contents

1. Identification & Management of Seed Borne Diseases
2. Seedborne Fungal Pathogens that Cause Important Diseases of Major Crops
3. Seedborne Diseases and their Importance
4. Diagnostic Methods for Seedborne Diseases
5. Detection of Seedborne Pathogens
Identification & Management of Seed Borne Diseases

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Department of Plant Science and Crop Protection
University of Nairobi
## Identification & Management of Seed borne Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Causal agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean anthracnose</td>
<td>Colletotrichum lindemuthianum</td>
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<tr>
<td>Halo blight (bean)</td>
<td>Pseudomonas savastanoi phaseolicola</td>
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<tr>
<td>Common bacterial blight (bean)</td>
<td>Xanthomonas axonopodis phaseoli</td>
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<tr>
<td>Bean common mosaic</td>
<td>Bean common mosaic virus</td>
</tr>
<tr>
<td>Head smut (maize)</td>
<td>Sphacelotheca reiliana, Ustilago maydis</td>
</tr>
<tr>
<td>Gray leaf spot (Maize)</td>
<td>Cercospora zea-maydis</td>
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<tr>
<td>Maize leaf blight</td>
<td>Drechslera turcicum</td>
</tr>
<tr>
<td>Stalk rot / ear rot (maize)</td>
<td>Fusarium graminearum, F. verticillioides, F. proliferatum, F. subglutinans, Stenocarpella maydis</td>
</tr>
<tr>
<td>Bacterial blight (cow pea)</td>
<td>Xanthomonas campestris vignicola</td>
</tr>
<tr>
<td>Sclerotinia wilt &amp; head rot (sunflower)</td>
<td>Sclerotinia sclerotiorum</td>
</tr>
<tr>
<td>Botrytis head rot (sunflower)</td>
<td>Botrytis cinerea</td>
</tr>
</tbody>
</table>

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Identification & Management of Seed borne Diseases

Seed discolouration, Shrivelling, rotting & reduced size

Reduced seedling vigour

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How does seed contamination occur?
<table>
<thead>
<tr>
<th>Seed contamination or infestation</th>
<th>Accompanying contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogen itself or parts of it stick or mix with seeds during:</td>
<td>Physical mixing of the seed with pathogen’s propagation organs</td>
</tr>
<tr>
<td>• Harvesting</td>
<td>• Spores</td>
</tr>
<tr>
<td>• Extraction</td>
<td>• Sclerotium</td>
</tr>
<tr>
<td>• Threshing</td>
<td>• Nematode’s galls</td>
</tr>
<tr>
<td>• Selection</td>
<td>• Contaminated plant parts or soil particles containing pathogens</td>
</tr>
</tbody>
</table>
Location of pathogen in seed

- Infection of the embryo
- Under the seed coat
- In the endosperm or cotyledon
- On the surface of seed

How pathogens infect seed

- Systemic Infection of the Seed
- Through flowers, fruits or funiculus
- Through the stigma
- Through the wall of the ovary or immature seed covers
- Through wounds & natural openings
Routes of active seed infection

- stigma
- style
- pollen tube
- ovary wall (pericarp)
- ovule (seed)
- nucellus
- egg sac
- testa (seed coat)
- egg cell (embryo)
- micropyle
- vascular trace
- funicle (funicular scar = hilum)

(from Maude, 1996)
Routes of active seed infection

A. Penetration through ovary wall

B. Systemic infection via vascular system

C. Penetration through floral parts

E.g.: Cladosporium variabile (spinach), Botrytis spp. (onion)

E.g.: Vascular wilt fungi, endophytes

E.g.: Ustilago nuda (grains), Cucumber mosaic virus

From Maude (1996)
Identification & Management of Seed borne Diseases

Maize Lethal Necrosis Disease

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Maize

Loose smut

Head Smut
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Maize leaf blight

Gray leaf spot

Maize rust

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Fusarium stalk rot of maize

Charcoal rot

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Diplodia stalk and ear rot of maize
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Ear rot of maize

Fusarium ear rot

Diplodia

Fusarium ear rot

Trichoderma

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Sorghum

Anthracnose
Helminthosporium leaf blight
Target spot
Head blight

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Smut on wheat ears

Wheat kernels with smut symptoms
Identification & Management of Seed borne Diseases

- Wheat
  - Loose smut
  - Stem rust
  - Leaf rust

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Diseases in seed crop production

- **Powdery mildew**
- **Barley yellow dwarf**
Diseases in seed crop production

Rice blast

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Bean anthracnose on pods and leaves
Angular leaf spot on bean
Identification & Management of Seed borne Diseases

Sclerotinia on bean stems and pods

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Aschochytta leaf spot  Web blight  Bean rust

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Root rots

Beans

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Halo blight on bean
Bean virus diseases
Virus diseases
Bacterial blight

Aschochyta

Cercospora

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Cowpea

Root rot

Rust

Anthracnose

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Identification & Management of Seed borne Diseases

Ground nut

Early leaf spot

late leaf spot

Alternaria leaf spot

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Groundnut

Rust

Aspergillus crown rot

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Ground nut rosette  

Virus diseases
Diseases in seed crop production

Green gram

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Sclerotinia Head Rot of sunflower
Shriveled and discoloured seed

Incubation rolled paper towel

Incubation between paper towel
Infected seeds
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Seeds infected with fungi

Bacterial isolated from infected seed

Seeds with symptoms of infection

Pure cultures of isolated pathogens

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MANAGEMENT OF SEED-BORNE DISEASES
Previous cropping

- Seed production fields should be free from volunteer plants to avoid contamination of the crop seed by:
  - Any seed which is difficult to remove from the crop seed
  - Cross-pollination;
  - Seed-borne diseases transmitted from volunteer plants
  - The previous cropping shall be such that there is the least possible risk of any soil borne diseases being present which could subsequently be transmitted in the harvested seed.
Production in disease-free areas

- Dry areas with low humidity (use irrigation)
- Bean anthracnose and Bacterial blights of bean
- Altering time of planting
- Crop isolation from other fields containing possibly diseased plants
Good production practices

- Use of certified seed
- Minimize plant stress - fertilization & watering
- Weed management
- Well-drained soils
- Seed rate - proper plant density to promote rapid drying of foliage
- Destroy/ plough under crop residues
- Proper crop handling (wash hands & implements)
- Removal of infected plants (roguing)
- Avoid working in field when wet
Identification & Management of Seed borne Diseases

Eradicate disease-causing pathogen from production area

- Remove alternate hosts and volunteer host plants
- Crop rotation
- Sanitation - residue management
- Creating conditions unfavourable to pathogens
- Seed treatment
- Use resistant/ tolerant crop varieties
- Use of disease-free planting materials
- Spray protective fungicides
- Control of Insect Vectors
Isolation and Field Inspection

- Seed crops should be isolated from all sources of pollen contamination and seed-borne diseases.
- Crop should be inspected at least once at appropriate stage of growth.
- At least 20% of the crop of Certified Seed should be inspected.
- Presence of any seed-borne disease should be at the lowest possible level.
Seed health testing
Germination test
Diseases in seed crop production

Seed health test for seedborne pathogens

Fast green test for physical damage

Tetrazolium test for seed viability

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## Identification & Management of Seed born Diseases

### Tolerated levels for seed borne diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Tolerance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head smut (maize)</td>
<td>2 plants per hectare</td>
</tr>
<tr>
<td>Loose smut (maize)</td>
<td>2 plants per hectare</td>
</tr>
<tr>
<td>Bunt (wheat)</td>
<td>1 head per 100 sq. m</td>
</tr>
<tr>
<td>Bunt (sorghum)</td>
<td>1 plant per 1,000 plants</td>
</tr>
<tr>
<td>Halo bight (bean)</td>
<td>None at inspection</td>
</tr>
<tr>
<td>Anthracnose (bean)</td>
<td>None at inspection</td>
</tr>
<tr>
<td>Common bacterial blight (bean)</td>
<td>None at inspection</td>
</tr>
<tr>
<td>Bean common mosaic</td>
<td>None at inspection</td>
</tr>
<tr>
<td>Bacterial blight (cow pea)</td>
<td>None at inspection</td>
</tr>
<tr>
<td>Botrytis head rot (sun flower)</td>
<td>5 plants per 1,000 plants</td>
</tr>
<tr>
<td>Sclerotinia wilt &amp; head rot (sun flower)</td>
<td>5 plants per 1,000 plants</td>
</tr>
</tbody>
</table>
THANK YOU
SEEDBORNE FUNGAL PATHOGENS THAT CAUSE IMPORTANT DISEASES OF MAJOR CROPS:

<table>
<thead>
<tr>
<th>PATHOGEN</th>
<th>CROP</th>
<th>DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria brassicicola</em></td>
<td><em>Brassica spp</em> (Crucifers)</td>
<td>Black spot</td>
</tr>
<tr>
<td><em>Mycosphaerella brassicicola</em></td>
<td><em>Brassica spp</em> (Crucifers)</td>
<td>Black ringspot</td>
</tr>
<tr>
<td><em>Peronospora parasitica</em></td>
<td><em>Brassica spp</em> (Crucifers)</td>
<td>Downy mildew</td>
</tr>
<tr>
<td><em>Phoma lingam</em> (Plenodomus lingam)</td>
<td><em>Brassica spp</em> (Crucifers)</td>
<td>Black leg</td>
</tr>
<tr>
<td><strong>PATHOGEN</strong></td>
<td><strong>CROP</strong></td>
<td><strong>DISEASE</strong></td>
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<tr>
<td><em>Alternaria porri</em></td>
<td><em>Allium cepa</em></td>
<td><strong>Purple blotch</strong></td>
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<tr>
<td></td>
<td>(onion)</td>
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<tr>
<td><em>Botrytis allii</em></td>
<td><em>Allium cepa</em></td>
<td><strong>Damping off, gray mold, neck rot</strong></td>
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<tr>
<td></td>
<td>(onion)</td>
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<tr>
<td><em>Cercospora arachidicola</em></td>
<td><em>Arachis hypogaea</em></td>
<td><strong>Leaf spot</strong> (Tikka disease)</td>
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<tr>
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<td>(Groundnut)</td>
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<tr>
<td><em>Cercospora personata</em></td>
<td><em>Arachis hypogaea</em></td>
<td><strong>Leaf spot</strong> (Tikka disease)</td>
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<td>(Groundnut)</td>
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<tr>
<td><em>Macrophomina phaseolina</em></td>
<td><em>Arachis hypogaea</em></td>
<td><strong>Root rot, stem rot</strong></td>
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<tr>
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<td>(Groundnut)</td>
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<tr>
<td><em>Alternaria alternata</em></td>
<td><em>Helianthus annus</em></td>
<td><strong>Seed rot</strong></td>
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<tr>
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<td>(Sunflower)</td>
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<tr>
<td><strong>PATHOGEN</strong></td>
<td><strong>CROP</strong></td>
<td><strong>DISEASE</strong></td>
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<tr>
<td><em>Alternaria</em></td>
<td><em>Helianthus annus</em></td>
<td>Blight</td>
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<td>zinniae</td>
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<td><em>Botrytis</em></td>
<td><em>Helianthus annus</em></td>
<td>Gray mold</td>
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<td>cinerea</td>
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<tr>
<td><em>Macrophomina</em></td>
<td><em>Helianthus annus</em></td>
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<td>phaseolina</td>
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<tr>
<td><em>Sclerotinia</em></td>
<td><em>Helianthus annus</em></td>
<td>White rot, wilt, stem rot</td>
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<td>sclerotiorum</td>
<td>(Sunflower)</td>
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<tr>
<td><em>Alternaria</em></td>
<td><em>Ricinus communis</em></td>
<td>mold, seedling blight</td>
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<td>Capsule</td>
<td>(Castor bean)</td>
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<td>ricini</td>
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<td>PATHOGEN</td>
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<tr>
<td>Ascochyta phaseolorum</td>
<td><em>Phaseolus vulgaris</em> (Bean)</td>
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<td><em>Colletotrichum lindemuthianum</em></td>
<td><em>P. vulgaris</em> (Bean)</td>
<td>Anthracnose</td>
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<td>Elsinoe phaseoli</td>
<td><em>P. vulgaris</em> (Bean)</td>
<td>Scab</td>
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<td><em>Fusarium oxysporum</em> f. sp. phaseoli</td>
<td><em>P. vulgaris</em> (Bean)</td>
<td>Wilt</td>
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<tr>
<td><em>Macrophomina phaseolina</em></td>
<td><em>P. vulgaris</em> (Bean)</td>
<td>Ashy stem blight, charcoal rot</td>
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<td>PATHOGEN</td>
<td>CROP</td>
<td>DISEASE</td>
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<tr>
<td><em>Phaeoisariopsis griseola</em></td>
<td><em>P. vulgaris</em> (Bean)</td>
<td>Angular leaf spot</td>
</tr>
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<td><em>Sclerotinia sclerotiorum</em></td>
<td><em>P. vulgaris</em> (Bean)</td>
<td>Sclerotial wilt</td>
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<td><em>Drechslera tritici-repentis</em></td>
<td><em>Triticum aestivum</em> (Wheat)</td>
<td>Leaf spot, yellow spot</td>
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<tr>
<td><em>Fusarium graminearum</em></td>
<td><em>Triticum aestivum</em> (Wheat)</td>
<td>Head blight, scab</td>
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<td><em>Septoria nodorum</em></td>
<td><em>Triticum aestivum</em> (Wheat)</td>
<td>Glume blotch</td>
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<tr>
<td>Septoria tritici</td>
<td>Triticum aestivum (Wheat)</td>
<td>Speckled leaf spot</td>
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<tr>
<td>Ustilago tritici</td>
<td>Triticum aestivum (Wheat)</td>
<td>Loose smut</td>
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<tr>
<td>Drechslera teres</td>
<td>Hordeum vulgare (Barley)</td>
<td>Net blotch</td>
</tr>
<tr>
<td>Rynchosporium secalis</td>
<td>Hordeum vulgare (Barley)</td>
<td>Scald</td>
</tr>
<tr>
<td>Claviceps fusiformis</td>
<td>Pennisetum typhoides (Pearl millet)</td>
<td>Ergot</td>
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<tr>
<td>PATHOGEN</td>
<td>CROP</td>
<td>DISEASE</td>
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<tr>
<td><em>Claviceps microcephala</em></td>
<td><em>Sorghum vulgare</em> (Sorghum)</td>
<td>Ergot</td>
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<td><em>Colletotrichum graminicola</em></td>
<td><em>S. vulgare</em> (Sorghum)</td>
<td>Anthracnose, red leaf, stalk rot</td>
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<tr>
<td><em>Fusarium moniliforme</em></td>
<td><em>S. vulgare</em> (Sorghum)</td>
<td>Seed rot</td>
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<td><em>Sclerospora sorghii</em></td>
<td><em>S. vulgare</em> (Sorghum)</td>
<td>Downy mildew</td>
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<td><em>Sphacelotheca cruenta</em></td>
<td><em>S. vulgare</em> (Sorghum)</td>
<td>Loose smut</td>
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<td>DISEASE</td>
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<tr>
<td><em>Sphacelotheca</em></td>
<td><em>S. vulgare</em></td>
<td>Covered smut, grain smut</td>
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<td><em>sorghi</em></td>
<td><em>(Sorghum)</em></td>
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<tr>
<td><em>Diplodia</em></td>
<td><em>Zea mays</em></td>
<td>Ear rot, seedling blight, stalk rot</td>
</tr>
<tr>
<td><em>maydis</em></td>
<td><em>(Maize)</em></td>
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<tr>
<td><em>Drechslera</em></td>
<td><em>Zea mays</em></td>
<td>Blight, southern leaf spot</td>
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<tr>
<td><em>maydis</em></td>
<td><em>(Maize)</em></td>
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<tr>
<td><em>Exserohilum</em></td>
<td><em>Zea mays</em></td>
<td>Blight-Northern leaf blight</td>
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<tr>
<td><em>turcicum</em></td>
<td><em>(Maize)</em></td>
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</tr>
<tr>
<td><em>(Syn. Drechslera</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>turcicum, Helminthosporium</em> turcicum)</td>
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<td>PATHOGEN</td>
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<tr>
<td><em>Fusarium roseum</em> (Syn. <em>Gibberella zeae</em>)</td>
<td><em>Zea mays</em> (Maize)</td>
<td>Pink ear rot</td>
</tr>
<tr>
<td><em>Fusarium moniliforme</em> (Syn. <em>Gibberella fujikuroi</em>)</td>
<td><em>Zea mays</em> (Maize)</td>
<td>Fusarium kernel rot</td>
</tr>
</tbody>
</table>
SEED BORNE DISEASES AND THEIR IMPORTANCE

Prof. A. W. Mwang’ombe / Dr. R. D. Narla
What are seed borne diseases?

- Seed-borne diseases are caused by pathogens such as fungi, bacteria, viruses and nematodes that live on the surface or interior of seed and have the potential to spread disease.

- All true/vegetative seed are infected by the above pathogens.

- Common seed borne pathogens
  - *Colletotrichum lindemuthianum* and *macrophomina phaseolina* (bean)
  - *Aspergillus flavus* (maize) & *A. parasiticus* (peanuts)
  - *Fusarium graminearum* (maize)
  - *Alternaria porri* (onion)
  - *Pyricularia oryzae* (rice)
  - *Bacteria - Xanthomonas campestris* (cabbage) *Ralstonia* in solanaceae
  - Nematode - *Aphelenchoides besseyi* (rice) - *Anguina tritici* (wheat)
  - Potato virus x
  - Bean common mosaic virus
  - Bean yellow mosaic virus
  - Maize dwarf mosaic virus
Some of the most important damages that pathogens can cause to seed are

1. Disease Transmission

Seed born pathogens transmit diseases between fields, regions and countries through seed and other planting material. For example, Diseases like Bacterial blight of paddy, Sclerotinia diseases of broad beans, common beans and recent cauliflower are transmitted through movement of improved seed.
• Importance of transmission can be realized when we know the seeding rate/ha (kg/ha), percentage seed infection and number of infected seeds/kg of seed.

E.g. Incase of Loose smut of wheat and Barley, with 0.1% seed infection bring 5000 infected seeds in a hectare of field. These 5000 give rise to equal number of infected plants (systemic) and in such cases yield losses are expected in the same ratio of 1:1
2. Complete Loss or Reduction in Seed Germination

Seed borne diseases/pathogens can be spread from the seed and infect the new plant in several ways.
- Upon sowing, moisture activates pathogens causing pre and post emergence damping off, eg: bean seeds infected with *Macrophomina phaseolina* cause 59% loss of germination. Soybean infected with *Cercospora kikuchii* – 12% loss of germination. Some of the pathogens like different species of *Fusarium, Pythium, Rhizoctonia, Sclerotinia, Altenaria* when also cause similar diseases in several other crops.
3. Seed abortion

Some of the seed borne pathogens like smut fungi in number of cereals and viruses like pigeon pea sterility mosaic virus cause heavy seed abortion resulting in 80-100% yield losses.
4. Reduction in seed quality

• Pathogen infections of seed often substantially reduce seed size resulting in weight reduction. Eg, leaf blight of sunflower – *Alternaria helianthi*, *A. zinnniae* infect the crop – severe leaf blight and yield loss of 80%

• Some other fungi – species of *Aspergillus*, *Fusarium*, infect standing maize causing seed rot.

• Sclerotisation, stromatisation and gall formation—*Claviceps fusiformis* – stromatisation of seed 60-70% yield losses in millets.

*Anguina tritici* in wheat causes seed galls.
• Seed discolouration – a very important and wide spread symptom produced on seed indicating presence of pathogen – *Cercospora kikuchii* – soy bean, *Fusarium moniliformae* – sorghum, *Aschochyta pisi* – Sweet pea all result in reduction in market value

• Infected seeds are at risk of being contaminated with mycotoxins and nutritional changes

• Biochemical changes in seed products – Groundnuts infected with *A. flavus* gives inferior quality of oil through reduction of the refractive index
5. Reduction in yield

Great yield losses are experienced worldwide through seed born pathogens.
• Fungal Seed borne Diseases
Anthracnose

- Caused by \textit{Colletotrichum lindemuthianum}

- The fungus is pathogenic to common bean, scarlet runner bean, mung bean, cowpea, and faba bean.
Fusarium root rot

- Caused by *Fusarium oxysporium*

- may complex with *Rhizoctonia solani* and *Pythium* spp.
Disease: Groundnut leaf spot
Causal agent: *Cercospora arachidicola*

- Disease symptoms - small chlorotic spots appear on leaflets 10 days after infection
- In five days, spots develop into mature, sporulating lesions
- Identification by morphology of conidiophores
Late leaf spot damage on groundnut
Rhizoctonia root rot

- Caused by the soilborne fungus *Rhizoctonia solani*. 
Southern blight

Caused by *Sclerotium rolfsii*

- Attacks a wide range of crops in Africa

Southern Blight on tomato stem
Angular leafspot caused by *Phaeoisariopsis griseola*
Fusarium headblight

- Causal agent: *Fusarium graminearum*
White mold

• Causal fungus *Sclerotinia sclerotiorum*.

• also known as Sclerotinia stem rot
Finger millet blast

*Pyricularia grisea*
• Bacterial Seed borne Diseases
Halo blight of beans caused by *P. (syringae) savastanoi pv phaseolicola* –
Bacterial blight of beans

Causal agent: *Xanthomonas axonopodis* pv. *phaseoli*
Fuscous Blight-
Xanthomonas axonopodis pv. phaseoli (syn. Xanthomonas campestris pv. Phaseoli) var. fuscans
Seed borne Nematodes
Wheat gall nematode *Anguina tritici*
White tip disease of rice by *Aphelenchoides besseyi*
Aphelenchoides besseyi
*Ditylenchus dipsaci* on faba beans causing stunting
*Ditylenchus dipsaci* on onions causing bulb rot
Ditylenchus dipsaci
Ditylenchus damage on maize
Seeds infected with Ditylenchus
Seed borne viruses
Bean Common Mosaic Virus
Pea seed borne mosaic virus
Tomato spotted wilt virus
"Global losses in food production due to seed born diseases are important negative factors in world agriculture"

- Total amount of annual global loss is equivalent to total amount of food need for the entire population of Latin America (with exception of storage fungi)
- Such waste cannot be accepted as a natural law
- Seed born diseases must be controlled"

Paul Neerguard

I am sure all of us as seed scientists and technologists appreciate these statements.
Seed Quality Assurance, Management and Control Processes

Diagnostic Methods For Seedborne Diseases

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Effects of Pathogen infection of seed

- A decrease in germinability
- Discoloration
- Biochemical changes
- Heating
- Mustiness and total decay
- Mycotoxin production
Location of Seedborne Pathogen

- Embryo
- Endosperm
- Seedcoat
- Surface borne
Diagnostic Methods for Seed borne Diseases

Objective of Seed Health

- Testing for Quarantine
- Testing for evaluation of planting value
- Testing for certification scheme
- Testing for advisability of seed treatment
- Testing seeds for storage quality of for feeding
- Testing for resistance of cultivars
Methods in Seed Health Testing
Methods in Seed Health Testing

- Visual examination of dry seed
- Seed washing test
- Blotter method
- Washing test
- Agar plate method
- Growing-on test
- Pathogenicity test
Inspection of dry seeds

- Provides quick information on insect, disease and mechanical damage to the seeds

**The fruiting structures of fungi**
- Acervuli, pycnidia, perictheicia, sclerotia on the seed surface or submerged in the seedcoat
- Sclerotia loosely mixed with seeds
- Individual spores or spore masses on the seed surface
Diagnostic Methods for Seed borne Diseases

Physical abnormalities include:

- Shriveling of the seed coat
- Reduction or increase in seed size
- Discoloration or spots in the seed coat
Seed washing test

- Applicable solely for seed born fungal and bacterial pathogens
- A known amount of seed in suspended in known amount of sterile saline (8.5% NaCl) overnight
- Extract is plated on agar medium and incubated
- Count number of colonies to determine CFU/seed for bacteria
Procedure

1. Transfer 50g seed taken from 1kg to Erlenmeyer flask and add 100 ml sterile water and 1 drop tween 20
2. Shake for 5 min, and sieve through cheese cloth for fungi
3. Transfer filtrate in to centrifuge tubes (1500-2000 rpm, 3min)
4. Pour off liquid and invert tubes
5. Add 1 drop of Shears solution and mount on a slide and observe under microscope (X100 -X400)
6. For bacteria, soak seeds in saline overnight; plate extract on agar medium
Diagnostic Methods for Seed borne Diseases

Blotter Method

Simple and inexpensive way to detect seedborne fungi

Procedure:

1. 9.5-cm Pyrex glass or clear plastic petri plates containing 2-3 layers of blotter papers moistened with distilled water.
2. Place seeds working sample equidistant on the petri plates
3. Incubate seeds at 22 °C under a 12-h light and 12-h dark cycle.

Results: Express results as a percentage infected seeds of the number of total seeds.
Agar plate method

- Detects and identifies seedborne fungi through colony characteristics which they exhibit when grown on nutrient agar.
- Media - water agar, potato dextrose agar, potato sucrose agar, Czapek-Dox agar, malt extract agar.
- Germination inhibitors - herbicide or sodium chloride

Procedure:

1. 400 seeds pretreated with 1% sodium hypochlorite for 10 min.
2. Place seeds agar media in 9.5-cm petri dishes.
3. Incubate at 22 °C for 5-8 days, either under alternate cycles of NUV light and darkness, or in darkness.
Results:

- Examine characteristic pathogen colonies, beginning on the third day and continuing through the eighth day of incubation.
- Also examine seeds under a stereo binocular microscope.
- View spores and other fungal structures under a compound microscope to distinguish the fungal forms.
- Express results as a percentage of seeds infected.
Diagnostic Methods for Seed borne Diseases

Plating

Purify

Microscopy

Chlamydospores

Spores

University of Nairobi, Kenya
Diagnostic Methods for Seed borne Diseases

**ISOLATION AND MICROSCOPY**

- **Epicoccum**
- **Penicillium**
- **Fusarium**
- **Alternaria**
- **Spores**
- **Chlamydospores**

University of Nairobi, Kenya
Paper towel method

- Seeds are submerged in a solution of 2.5% sodium hypochlorite for 5 min, rinsed in sterile distilled water and blotted dry.
- Spread the seeds in replicates of 50 on double sheets of wet paper towelling 350 x 450 mm.
- Cover seeds with one sheet of wet paper towelling.
- Fold the paper twice lengthways and cover it with a sheet of polythene to maintain the moisture during incubation.
• Incubate for 7 days at 20 °C in darkness.
• Examine seeds by naked eye for growth of fungi.
• Observe seeds under dissecting microscope for fungal structures.
• Mount fungal growth on microscope slides & observe under high-power microscopes (mag ×200)
Seedling symptom test

- Some of the seed-borne pathogens/obligates or deep seated infections cannot be grown on blotters or agar media.
- Detects seedborne fungal, viral, and bacterial pathogens which are readily transmittable
- Seed are planted either in sterile soil, sand or paper towels
a) Paper towel test

- Sterilized seeds are sown on paper towels, 1-2 cm apart depending on seed size. Seeds are rolled so that each seed is in an individual roll,
- Incubate for 2-3 weeks under sterile conditions providing appropriate relative humidity for seed germination & symptom development.
- Observe the symptoms and identify the pathogen.
b) Growing-on test

Detects seedborne fungal, viral, and bacterial pathogens which are readily transmittable.

Procedure:

- Sow seeds on a suitable medium (sterilized soil, sand, on paper towel or water agar) under optimal conditions for germination.
- Incubate under controlled conditions for seedlings to grow & develop symptoms.
- Observed characteristic symptoms, pathogens isolated & identified.
Detection of seedborne bacteria
e.g. Halo blight and common bacterial blight of bean

- Suspend seeds in sterile saline plus Tween 20 (0.02% v/v)
- Soak subsamples overnight (16-18 h) at 5 ± 4 °C).
- Shake on to obtain a homogenous extract.
- Prepare a tenfold dilution series from the seed extract.
- Plate each dilution & undiluted seed extract selective media.
- Incubate inverted plates and examine after 4-5 days
- Subculture suspect colonies to sectored plates of KB.
- Pathogenicity test of isolated bacteria by inoculation on cotyledons of bean seedlings of known susceptibility
Diagnostic Methods for Seed borne Diseases

Serial dilution in sterile distilled water

1ml
1ml
1ml
1ml
1ml

9ml

Saline extract

10^1
10^2
10^3
10^4
10^5

Plate 1ml of each dilution in molten agar medium. Incubate and count the number of colonies for each dilution. Determine bacterial population by multiplying the number of colonies by the dilution factor.

Soak overnight in sterile saline

University of Nairobi, Kenya
Pathogenicity test

1. Isolated bacteria
2. Inoculate on germinated bean cotyledons
3. Water soaking symptom
Apart from the above routine procedures, sophisticated techniques like ELISA, PCR also can be used to detect seed born pathogens.
THANK YOU FOR THE AUDIENCE
Detection of seed born Pathogens

Routine Testing Methods for Seed Health

Prof. A. W. Mwang’ombe/Narla, R.D. /Michael Starr
Seed health

Seed is usually tested to establish seed health status.

Seed health refers primarily to presence or absence of disease causing microorganisms such as fungi, bacteria, viruses, and animal pests such as nematodes and insects, but physiological conditions such as trace element deficiency may also be involved (International Rules of Seed Testing (ISTA, 1985)).
Why Seed testing?

- Seed testing is required to establish whether seed is infected.
- To detect the most important seed-borne pathogens.
- Testing seed before sowing identifies potential disease problems and allow steps to be taken to reduce the disease risk.
- Laboratory testing is usually required, as infected seed may have no visible disease symptoms.
**Why Seed testing?**

- Many crop diseases can be seed-borne and significant crop losses can result from the use of infected seed.
- Uncontrolled movement of infected seed between regions can result in the rapid expansion of the area affected by these diseases.
- Therefore, laboratory testing is usually required, as infected seed may often have no visible disease symptoms.
Detection of seed born pathogens is done by the following Methods

Non-incubation methods
1. Dry seed inspection (visual examination)
2. Seed washing test

Incubation methods
3. Blotter test
4. Agar test
5. Seedling symptom test
1. Dry seed inspection (Visual examination)

- A qualitative test that detects fungal/bacterial seed infection by discoloration in seed coat, abnormal size or shape
- Best for fungi producing visible structures like sclerotia, stromata etc
- Detects insect/mechanical damage
- Useful for purity analysis (weed and any other seed contamination, stones, etc)
Dry seed inspection procedure

1. Acquire a sample
2. Inspect all seed parts carefully with naked eye and remove, identify non-seed matters
3. Carefully examine for seed galls, sclerotia and smut balls
4. Using hand held lens, examine for presence of discoloration and fungal structures, spores or spore deposits adhering on seed coat.
2. Seed washing test

- Applicable solely for seed born fungal pathogens

- A known amount of seed in suspended in known amount of sterile distilled water

Washing test seed assay
Washing test procedure

1. Transfer 50g seed taken from 1kg to Erlenmeyer flask and add 100 ml water and 1 drop tween 20
2. Shake for 5 min, and sieve through cheese cloth
3. Transfer filtrate in to centrifuge tubes (1500-2000 rpm, 3min)
4. Pour off liquid and invert tubes
5. Add 1 drop of Shears solution and mount on a slide and observe under microscope (X100 –X400)
3. Blotter test

- Seeds are incubated for 7 days at 20-22 °C.
- Fungi associated with the seeds are then examined and identified under microscope.
Blotter test procedure

- Line petri dishes with 3 filter papers (blotters) sterile, soaked in distilled water
- Spread seeds in Petri dishes at regular intervals (10 or 25/dish)
- Incubate at 20-22 °C for 7 days in alternating cycles of 12hrs light/darkness using near ultraviolet (NUV)
- Examine seeds after 7 days under microscope and identify the pathogens
4. Agar test

- Seed borne fungi are also detected and identified based on characters of colonies on agar directly developing from seed.
Preparation of the agar media

- Calculate the amount of agar medium for testing e.g. 400 seeds of a sample. The amount of agar will depend on the number of seeds to be plated in each petri dish (10 small sizes sees per dish, e.g. rice and 5 large sized seeds per dish, e.g. beans, soybeans).

- Sterilize agar medium in conical flasks or in Pyrex bottles if required add 0.3 g streptomycin sulphate in 1000 ml agar.

- Before pouring, let the agar medium cool down to around 50°C. Add antibiotic in the agar medium, if required e.g. 0.3 g streptomycin sulphate in 1000 ml agar.

- Since streptomycin sulphate is toxic, wear gloves while weighing and pouring it into the molten agar medium.

- Pour the medium in sterile petri dishes, approximately 15 ml per dish. Pouring should be done on a clean table room which has been decontaminated e.g. in a LaminarAir flow bench. Let the dishes solidify completely before plating seeds.
**Agar test procedure**

1. Surface sterilize the seeds
2. Plate seeds on agar on petri plate using sterile forceps
3. Incubate for 7-10 days
4. Observe the plates for fungal colonies from day 2 onwards
5. Observe colonies under microscope
6. Fungi are identified based on colony characteristics
7. Percentage of infections is calculated
8. For bacterial isolations, nutrient agar is used.
5. Seedling symptom test

Some of the seed-borne pathogens/obligates or deep seated infections cannot be grown on blotters or agar media. Therefore, seed has to be planted either in sterile soil or paper towels.

When these are provided normal conditions for seed germination, after days of incubation, seeds germinate and if infected, produce characteristic symptoms of the pathogen. These effects can be seen if seeds are sown on suitable substrate and seedling grown under environmental conditions which support expression of such effects.
Seedling symptom test procedures

1. Paper towel test

Sterilised seeds are sown on paper towels, 1-2cm apart depending on seed size. These seeds are rolled so that each seed is in an individual roll, then incubated for 2-3 weeks under sterile conditions providing appropriate relative humidity for seed germination and symptom development.

Observe the symptoms and identify the pathogen
Seedling symptom test procedure

2. Growing on test

Seeds are in sterile soil either in individual pots or seed trays and appropriate conditions for seed germination are provided. Pots are incubated under controlled conditions for seedlings to grow and develop symptoms. Symptoms are observed, pathogens isolated and identified.

Apart from the above routine procedures, sophisticated techniques like ELISA, PCR also can be used to detect seed born pathogens.