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Seed Mycoflora and Seed Health Testing

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Introduction

Seed is the basic unit for production of the world's food crop. In recent years seed has become an international commodity which are exchanged for germplasm around the world. Seed is, however, also an efficient means of introducing plant pathogens into a new area as well as providing a means of their survival from one cropping season to another. Seed health is a well-recognized factor in the modern agricultural science for desired plant population and good harvest. Fungi which survive in seed are one of the most important biotic constrains in seed production worldwide. They are responsible for both pre- and post-emergence death of grains, affect seedling vigour, and thus cause some reduction in germination and also variation in plant morphology. Fungi outnumber all other types of pathogens that attack plants and cause a very severe economic impact on agricultural production due to their ability to induce diseases of cultivated crops that result in important yield losses.

Three primary organizations which publish standardized seed health test methods for use in international trade are: International Seed Testing Association (ISTA), International Seed Health Initiative (ISHI), and in the United States, the National Seed Health System (NSHS).

Seed Health Testing and Detection

2.1. Seed Health Testing- Seed testing is needed for a numerous reason: to determine the quality of seed based on several seed quality attributes; to provide a basis for price & consumer

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discrimination among seed lots and seed sources; to determine the source of a seed problem, thereby facilitating any corrective measure(s) that may be required; and to fulfil legal and regulatory requirements for certified seed classes and allow for seed movement across international boundaries. Generally seed health tests have been categorised into the following four different groups based on the general methods used to observe the target pathogen. Such as Direct Inspection, Incubation Tests, Examination of the embryo (embryo count method), Immunoassays and Molecular Methods.

Fig. 1. Infected Seeds

2.1.1. Direct examination

Direct inspection of dry seed is a qualitative and semi-quantitative seed health testing method where both the fruiting structures of fungi are noticed under stereomicroscope or effects of fungal pathogens on the physical appearance of the seed are seen. By this method, it is possible to detect sclerotia, smut balls, fungal spores, and other fructifications such as pycnidia, perithecia, etc. If seeds are severely infested by some organisms, they may be reduced in size or discoloured.

2.1.2. Incubation tests

The seeds will be incubated for a certain period in the agar plate or blotter test, under a specific environmental condition to allow pathogens on the seed to grow. Different fungi are identified by features such as the form, length and arrangement of conidiophores, size and septation and chain formation of conidia.

2.1.3. Blotter tests/Seedling symptom tests

Blotter tests are like germination tests in that seeds are placed on moistened layers of blotter paper and incubated under 1conditions that promote fungal growth. The seed may then be allowed to germinate, and fungal seed-borne infections may manifest themselves by any pertinent signs.

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2.1.4. Agar plate

Agar plate is the most common method used for identification of seed borne fungi. Incubation methods allow the detection of viable fungus material even at the preliminary phase of development of the fungus.

Fig. 2. Blotter method

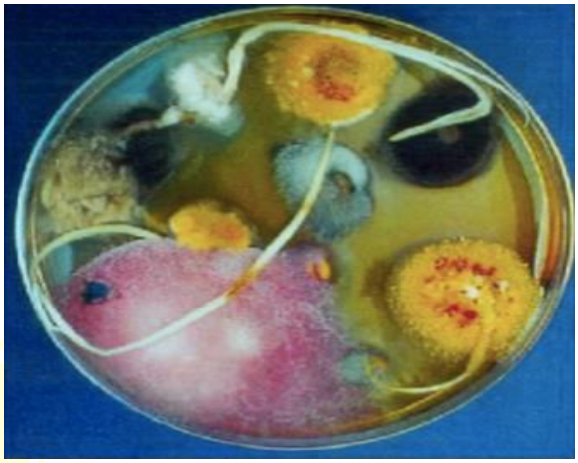

Fig. 3. Agar Plate Method

2.1.5. Examination of the embryo (Embryo count method)

Staining methods are used for seed borne pathogens which cannot be detected by direct inspection or incubation methods. The standard method used in seed health testing is that of staining of barley embryos for the presence of loose smut (*Ustilago segetum var. tritici*) mycelium.

2.1.6. Immunoassays

Immunoassays present a more sophisticated approach to testing, with Enzyme Linked Immunosorbent Assays (ELISA) and immunofluorescence being most common. For example, soybean mosaic virus, bean pod mottle and other viruses can be detected using ELISA.

2.1.7. Molecular methods

Molecular biology methods for agricultural diagnostics have become an area of increasing interest recently. There are DNA-based molecular techniques, the most common being the polymerase chain reaction (PCR), which selectively increases pathogen DNA.

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2.2. Seed Health Detection Methods

Detection methods deals with establishing the presence of a particular target organism within a sample, with special emphasis on symptomless individuals. In general seed health detection can be classified in to two major assays, such as the conventional seed detection assays and polymerase chain reaction-based seed detection assays.

2.2.1. Conventional seed detection assays

Seed assays have been developed based on different technologies including visual examination; selective media; seedling grow-out tests and serological techniques.

2.2.1.1. Bioassays

One of the oldest seed health assays is the grow-out. Its sensitivity is less assured, as inoculation thresholds may differ depending on the plant cultivar being tested, variations in environmental conditions, fertility, and other factors. Because grow-outs rely on symptom expression, a positive result usually is irrefutable evidence that the bacterium was present, viable and pathogenic.

2.2.1.2. Serological methods (Immunoassays)

Serological seed assays rely on antibodies (polyclonal or monoclonal) generated against unique antigens on the surfaces of plant pathogens. Antibodies bind strongly and specifically to their antigens and can then be detected by the enzymatic digestion of substrates or fluorescent tags. Serological methods used to detect and identify bacterial pathogens include agglutination tests, immunofluorescence microscopy (IF), immunofluorescence colony-staining (IFC), enzyme-linked immunosorbent assays (ELISA), Western blot, lateral flow devices (e.g., immunostrips), flow cytometry, and immunocapture techniques such as immunomagnetic separation (IMS).

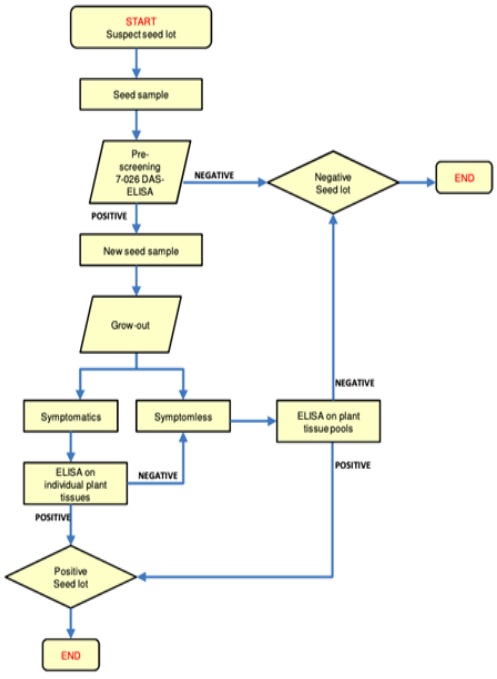

Fig. 4. Grow out test and ELISA

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2.2.2. Nucleic Acid–Based Detection Methods

Polymerase chain reaction (PCR) is the in-vitro, primer-directed, enzymatic amplification of nucleic acids. This technique has been used in many diverse applications including diagnosis of plant diseases. Although PCR is a sensitive technique and, theoretically, capable of detecting a single bacterial cell, the sample size and volume of seeds (e.g., 30,000 seeds/litre of buffer) being tested in conjunction with the small volume (~4 µl) that can be used as template in the PCR reaction make PCR no more sensitive than many other techniques. Nested PCR increases sensitivity by utilizing a second round of amplification using primers designed to anneal to internal regions of the amplicon produced by the first round of amplification. Using nested PCR, detection of *Ralstonia solanacearum*, the causal agent of bacterial wilt was carried out in tomato seeds.

2.2.2.1. Use of nucleic acid–based methods in epidemiology research on seedborne pathogens

These techniques can be applied to recognise the sources of seedborne infections, the location of pathogens within seed tissues to confirm the occurrence of seed transmission and its mechanisms and to understand the influence of external biotic and abiotic factors on seed transmission or other phases of the disease cycle. One example is watermelon fruit blotch (*A. avenae* mutants. *citrulli*), where the use of IMS-PCR facilitated the detection of a high incidence of infection in seeds from symptom less fruit following blossom inoculation.

2.2.2.2. Use of markers in seedborne pathogens

The most employed types of markers have been naturally occurring genetic markers, including antibiotic resistance, vegetative compatibility, and molecular markers unrelated to phenotype. Antibiotic resistance in bacterial pathogens, naturally occurring, induced through mutation, or inserted by genetic engineering, has been used effectively as a marker for decades. The contribution of seedborne inoculum to epidemics of *Stagonospora* leaf blotch was characterized by using *Stagonospora nodorum* strains identifiable by unique AFLP profiles. Mycotoxin production is another genetic marker used to investigate the importance of seedborne *F. verticillioides*.

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Diagnostic method	Time required	Sensitivity	Specificity	Ease of implementation	Examples of pathogens detected[a]
Visual examination	Very high	Low	Low	Mycological skills required	*Phomopsis* spp., *Cercospora kikuchii*, *Peronospora manshurica*/soybean seed; *Cylindrocladium parasiticum*/peanut seed; *Colletotrichum dematium*/chilli seed; *Septoria apii*/celery seed
Seed washing technique	Very high	Low	Low	Mycological skills required	*Peronospora manshurica*/soybean seed
Freeze blotter incubation	Low	Low/moderate	Moderate	Mycological skills required	*Alternaria dauci*, *Alternaria radicina*/carrot seed; *Leptosphaeria maculans*/ Brassicaceae seed
Agar medium incubation	Low	Low/moderate	Moderate	Mycological skills required	*Alternaria dauci*, *Alternaria radicina*, *Alternaria carotiincultae*/carrot seed; *Verticillium dahliae*, *Fusarium* spp./ Cucurbitaceae seed; *Botrytis* spp./onion seed
Serology-based assay	High	Moderate/high	Moderate/high	Ease of interpretation	*Macrophomina phaseolina*/cowpea seed
Conventional PCR	Moderate/high	High	High	Molecular biology skills required, ease of interpretation	*Alternaria brassicae*, *Leptosphaeria maculans*/Brassicaceae seed; *Ascochyta lentis*/lentil seed; *Alternaria radicina*/carrot seed; *Phoma valerianella*/lamb's lettuce seed; *Fusarium oxysporum* f. sp. *basilici*/basil seed
BIO-PCR[b]	Moderate	Very high	High	Molecular biology skills required, ease of interpretation	*Alternaria dauci*, *Alternaria radicina*/carrot seed; *Alternaria brassicae*, *Leptosphaeria maculans*/Brassicaceae seed; *Ascochyta rabiei*/chickpea seed; *Fusarium oxysporum* f. sp. *lactucae*/ lettuce seed
Nested PCR	Moderate	Very high	High	Molecular biology skills required, ease of interpretation	*Colletotrichum lindemuthianum*/bean seeds; *Fusarium oxysporum* f. sp. *lactucae*/lettuce seeds
Real-time PCR	High	Very high	High	Molecular biology skills required	*Alternaria brassicae*, *Plasmodiophora brassicae*/Brassicaceae seed; *Didymella bryoniae*/Cucurbitaceae seed; *Botrytis* spp./onion seed; *Verticillium dahliae*/ spinach seed; *Colletotrichum lindemuthianum*/bean seed; *Fusarium oxysporum* f. sp. *basilici*/basil seed
MCH-PCR[c]	High	Very high	High	Molecular biology skills required	*Didymella bryoniae*/Cucurbitaceae seed; *Botrytis* spp./onion seed
Laser biospeckle technique	High	High	High	Technological skills required	*Fusarium oxysporum*, *Aspergillus flavus*, *Sclerotinia* spp./bean seed
Videometer	High	High	High	Technological skills required	*Stemphylium botryosum*, *Cladosporium* spp., *Fusarium* spp., *Verticillium* spp., *Alternaria alternata*/spinach seed

Table. 1. Features of different method of assays

CONCLUSION

Seed is a small embryonic plant which is a basic unit of production for the world's food crop. It is an efficient means of introducing plant pathogens into a new area as well as providing a means of their survival from one cropping season to another. Seed health is a well-recognized factor in the modern agricultural science for desired plant population and good harvest. Seed-borne fungi are one of the most significant biotic constrains in seed production worldwide. ISTA, ISHI and NSHS are three primary organizations that publish standardized seed health test methods. Seed health testing which helps in detecting seed-borne pathogens is a main step in the management of crop diseases. Since seed serve as means of dispersal and survival of plant pathogens, it is critical to test its health before using it as planting material. Thus, seed health testing and detection is a first line methodology in management of seedborne diseases in plants.