

# AGRI MAGAZINE

(International E-Magazine for Agricultural Articles)
Volume: 02, Issue: 07 (July, 2025)

Available online at http://www.agrimagazine.in

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## Cutting-Edge Approaches in Seed Borne Pathogen Detection \*Sumayya Mullal<sup>1</sup> and Vinayak Savalagi<sup>2</sup>

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Seed is a small embryonic plant which is an effective means of introducing plant pathogens into a new zone as well as providing a way for their survival from one cropping season to another. Seed health is a well-recognized feature in the modern agricultural science for wanted plant population and good harvest. Seed-borne fungi are one of the most significant biotic constrains in seed production international. Seed health testing to detect seed-borne pathogens is a vital step in the management of plant diseases. Seed health is a measure of liberty of seeds from pathogens. ISTA, ISHI and NSHS are three chief organizations that publish standardized seed health test procedures. Seed Pathology is the learning of seedborne diseases and pathogens. About 90 per cent of all edible crops grown on globe are propagated by seed. Many plants are attacked by dreadful seed-borne diseases. Seed health refers to the presence or nonappearance of disease-causing organisms such as fungi, nematodes, bacteria, viruses besides insects and to the status of seeds in seed lot.

Seed quality can be compromised by the presence of non-disease-causing contaminants within a seed lot. These contaminants include weed seeds that compete with the target seeds for nutrients, as well as other seeds, plant debris, soil particles, and overwintering insect eggs, all of which can degrade seed lot quality. The impact of seed pathogens can lead to reduced crop yields, diminished germination and vigor, the development of plant diseases, seed discoloration and shriveling, biochemical changes in seeds, and alterations in physical seed properties. Plant diseases contribute to an estimated 12 percent loss of potential global production, translating to approximately \$50 billion annually. Employing various seed health testing methods can aid in the identification and management of seed-borne pathogens, helping to mitigate these losses.

Kandan *et al.* (2016) conducted a study using the Loop-Mediated Isothermal Amplification (LAMP) assay to detect latent infections of *Colletotrichum capsici* in chilli seeds and compared its performance with traditional PCR-based methods. Their results demonstrated that the LAMP assay is highly effective for the early detection and identification of *Colletotrichum capsici*, offering a simple and rapid diagnostic tool for identifying infected chili plants in agricultural fields and quarantine laboratories.

Khan *et al.* (2018) compared LAMP and PCR-based assays for the rapid detection of *Alternaria solani*. They found that the LAMP assay could successfully amplify diluted pure *Alternaria solani* cultures, providing a more sensitive and quicker visual detection method compared to conventional PCR strategies. This technique allows for early disease prediction and helps mitigate the risk of epidemics.

Awad *et al.* (2019) explored the use of nanoparticles in DNA extraction and advanced PCR techniques for detecting seed-borne pathogens. They found that silver nanoparticles significantly increased the DNA yield and PCR product from *Colletotrichum maydis* infected maize seeds compared to other extraction methods. The silver nanoparticles enhanced the

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electrical conductivity and chemical stability of DNA, leading to more efficient amplification of DNA strands.

Torre *et al.* (2020) compared DAS-ELISA and RT-qPCR for detecting cucumber green mottle mosaic virus (CGMMV), squash mosaic virus (SqMV), and melon necrotic spot virus (MNSV). The RT-qPCR assays proved to be highly sensitive for evaluating seed lots, particularly for CGMMV and SqMV. The study suggests that RT-qPCR could be adopted in seed certification programs as either a replacement for or complement to the existing DAS-ELISA method.

#### Seed health testing methods **Examination of ungerminated seeds Incubation test** Molecular methods PCR . Inspection of dry seed Blotter method Real time PCR Examination of soaked 2,4-D method Real time PCR Freezing method using specific Examination of Agar plate flouresent suspension obtained method probes from washing of seeds Water agar plate Reverse and sedimentation method transcriptase Examination of whole Seedling **PCR** embryo symptom test **Nested PCR Bio-assays and biochemical tests** Inspection of plants in field BIO-PCR DNA-chips (Micro array Indicator plant test Field trails Phase-plaque test Inspection of seed crop Serological test Electron microscopy Molecular methods: ELISA, PCR (Tsedaley, 2015)

Fig.1: Classification of different seed health testing methods

Jitesh and Bijender (2022) investigated methods for detecting seed-borne fungal pathogens associated with seed discoloration in rice using the agar plate method and the standard blotter method. Their findings indicated that the agar plate method was more effective than the standard blotter test for pathogen detection.

Table 1: Comparison of different methods for seed health testing

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Assay Time of Specificity	Time of required	Sensitivity	Ease of application	Specificity
Visual examination	5–10 min	Low	Simple	low
Semiselective media	2–14 d	Moderate	Simple	Low- moderate
Seedling grow-out assay	2–3 weeks	Low	Simple	Moderate- low
Conventional DNA extraction and polymerase chain reaction (PCR)	5–6 h	High	Simple	High –very high
Serology-based detection	2-4 h	Moderate- high	Simple	Moderate- high
BIO-PCR	2-3 d	Very high	complicated	Very high
Real-time PCR	40-60 min	Very high	complicated	Very high
DNA microarray	6 h	Very high	complicated	Very high

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## **Conclusion**

When selecting seed health testing methods, key criteria include specificity, sensitivity, speed, simplicity, cost-effectiveness, and reliability. PCR (Polymerase Chain Reaction) offers many advantages that make it highly suitable for detecting seed-borne pathogens. Various seed health testing methods can help identify sources of seed-borne infections, locate pathogens within seed tissues, confirm seed transmission, and understand how external biotic and abiotic factors affect seed transmission and other stages of the disease cycle. Since seeds are crucial for the dispersal and survival of plant pathogens, it is essential to assess their health before use as planting material. Effective seed health testing is a crucial first step in managing seed-borne plant diseases.

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