Seed Health: Testing and Management

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Abstract

Healthy seeds play an important role in growing a healthy crop. Seed health testing is performed by detecting the presence or absence of insect infestation and seed-borne diseases caused by fungi, bacteria, and viruses. The most detrimental effect of seed-borne pathogens is the contamination of previously disease-free areas and the spread of new diseases. Sowing contaminated or infected seeds not only spreads pathogens but can also reduce yields significantly by 15–90%. Some of the major seed-borne diseases affecting yield in cereals, oilseeds, legumes, and vegetables, particularly in the warm and humid conditions prevailing in the tropical and sub-tropical regions, are blast and brown spot of rice, white tip nematode and ear-cockle in wheat, bacterial leaf blight of rice, downy mildews, smuts, head mould, seedling rots, anthracnose, halo blight, and a number of viral diseases. Hence, detection of seed-borne pathogens, such as fungi (anthracnose, bunt, smut, galls, fungal blights), bacteria (bacterial blights, fruit rots, cankers), viruses (crinkle, mottle, mosaic), and nematodes (galls and white tip), which transmit through infected seed to the main crop, is an important step in the management strategies for seed-borne diseases. Thus, seed health testing forms an essential part of seed certification, phytosanitary certification, and quarantine programmes at national and international levels. Detection of seed-borne/transmitted pathogens is also vital in ensuring the health of the basic stock used for seed production and in maintaining the plant germplasm for future research and product development. Besides the precise and reproducible testing methods,

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appropriate practices during seed production and post-harvest handling, including seed treatment and storage, are important components of seed health management and sustainable crop protection.

Keywords

Seed health testing methods · Seed-borne diseases · Management of SBDs · Seed treatment

1 Significance of Seed Health Testing

Seed health is an essential component of seed quality. It is estimated that 30% of seed-borne diseases can be controlled by using disease-free seeds. For many of these diseases, fungicides are not available or registered, and resistant cultivars are not available, necessitating the use of disease-free seed as the only means of crop protection. Hence, research and development priorities to facilitate and improve the scope of seed health testing need special attention.

Seeds are regularly moved internationally, in small or large quantities, for trade and research purposes. These are often produced in one or more countries and distributed from those to several other countries. Seed health issues are becoming increasingly important in the international seed trade. With the advent of free trade, many countries are redefining their phytosanitary requirements to prevent the introduction of new and harmful pathogens into their countries (McGee 1997). To provide scientific answers to the problems encountered in the worldwide movement of seeds, an internationally accepted programme is needed to standardize seed health tests and inspection practices.

Development of seed health testing methods needs to be viewed in light of the general evolution of the seed sector. The importance of seed health can be established by the extent of losses attributed to seed-borne pathogens (Mathur et al. 1988; Mekonnen Gebeyaw 2020), which could be as high as 15–90%. Hence, predictive relationships need to be established between the seed-borne pathogens which cause significant yield loss (Hajihasani et al. 2012), and reliable, effective, inexpensive, and rapid detection methods need to be standardized for detecting the same.

Irrespective of the detection methodology, the specificity, sensitivity, reliability, and efficiency of the assay and pathogen tolerance in the seed lot also need to be understood before a technique is considered acceptable for seed health test as a tool for disease management and can be routinely used in seed quality assessment. These considerations also help develop national policies and methods for conducting seed health tests as part of crop protection to increase crop yields.

The purpose of seed health testing may be any of the following:

- Testing for seed certification schemes.
- Testing to make accurate decisions regarding the appropriate use of seed treatment.
- Testing for quarantine purposes to avoid the spread of disease to new regions and to issue a phytosanitary certificate.
- Testing for the evaluation of planting value in the field.
- Testing for treated seeds.
- Testing for storage quality.
- Testing to assess the prevalence of seed-borne infection or the importance of a seed-borne disease in a research programme.
- Testing seeds for resistance of cultivars.

2 Seed Sampling

Seed sampling is the key to obtain accurate seed health test results. The low frequencies of the many important seed-borne pathogens in and on the seed and also the low to very low seed infection thresholds may cause disease outbreaks leading to huge economic losses. Therefore, seed sampling for seed health testing needs special attention. A seed sample should be the representative of the entire seed lot. Seed health tests are nearly always performed on a sample drawn from the seed lot which may be as large as 10,000–30,000 kg. It is, therefore, critical that the samples used for testing are reliable representatives of the seed lot, and this requires standardized sampling procedures. Seed testing organizations such as the International Seed Testing Association (ISTA) and the Association of Official Seed Analysts (AOSA) have developed specific rules and procedures for seed sampling for the evaluation of seed quality traits like germination and physical purity and seed health testing.

The objective of seed sampling, in particular reference to seed health testing, as per the ISTA Rules is 'to obtain a sample of a size suitable for tests, through which the probability of an infection being present is established only by its level of occurrence within the seed lot'. The two basic considerations of seed sampling are firstly to obtain a test sample that accurately represents the composition of the seed lot as a whole and secondly to keep in mind that regardless of how accurately an analysis is performed, the results represent only the standard of the sample submitted for analysis.

The general procedure for the seed sampling method is shown in Fig. 1 and is in accordance with AOSA and ISTA procedures. Primary samples of equal size are taken randomly from the whole seed lot and combined and blended to make a homogeneous composite sample. The submitted sample, which is distributed to the testing facility, is drawn from the composite sample. The working sample, which is used for performing the tests, is obtained from the submitted sample after prescribed blending and dividing to ensure sample homogeneity. In some cases, the working sample may be the entire submitted sample or a composite sample. These

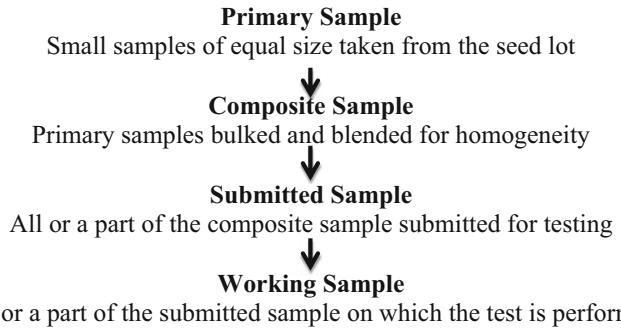

Fig. 1 Procedure for seed sampling for the evaluation of seed quality, including seed health testing

sampling schemes are designed to reduce variability and ensure uniformity of working samples.

3 Genesis of Seed Health Testing

It was Frederick Nobbe, under whose leadership the first seed testing laboratory was established in 1869 in Tharandt, Germany, and this was followed by the second lab in 1871, in Copenhagen, Denmark, under the guidance of E. Moller Holst. Subsequently, seed testing spread rapidly in Europe during the next 20–30 years. By the beginning of the twentieth century, around 130 seed testing stations were operating in Europe. In the United States, the first seed testing laboratory was opened in 1876. The first seed health testing laboratory was established in 1918 at the Government Seed Testing Laboratory in Wageningen, the Netherlands. In India, currently notified Central Seed Testing Laboratory (CSTL), at the Indian Agricultural Research Institute (IARI) New Delhi, is the first seed testing laboratory, established in 1961 (Jha 1993).

Dorph-Petersen (1921) who later became the ISTA president, in a report entitled 'Remarks on the Investigations of the Purity of Strain and Freedom from Disease', described the field trials for cultivar purity and detection of stripe (*Fusarium*) and smut diseases. The International Seed Testing Association was formed at the 1924 Congress, and one of its first technical committees was aimed at *Investigations of Genuineness of Variety and of Plant Diseases*, the forerunner of the *Plant Disease Committee* (PDC). The first *International Rules for Seed Testing* was published by ISTA in 1928, which contained a special section on *Sanitary Condition* with special mention of *Claviceps purpurea, Fusarium, Tilletia,* and *Ustilago hordei* on cereals; *Ascochyta pisi* on peas; *Colletotrichum lindemuthianum* on beans; and *Botrytis, Colletotrichum linicola,* and *Aureobasidium lini* on flax (Wold 1983).

The Plant Disease Committee of ISTA in 1957 established a comparative seed health testing programme aimed at standardizing techniques for the detection of seed-borne pathogens (Mathur and Jorgensen 2002). In 1981, the referee groups

were re-organized into crop groups with working groups conducting comparative tests on seeds of beet, crucifers, legumes, temperate cereals and grasses, tropical and sub-tropical crops, and viruses.

The Seed Health Committee (formerly the Plant Disease Committee) started the development of Guidelines for Comparative Testing of Methods for the Detection of Seed-borne Pathogens in 1993. This resulted in a complete revision of the method validation process for seed health testing as described in the Handbook of Method Validation. The methods have been validated and included in the Annexe to Chap. 7 of the International Rules for Seed Testing. The aim of the Seed Health Committee has been to develop and publish the validated procedures for seed health testing and to promote uniform application of these procedures for the evaluation of seeds moving in international trade (Hampton 2005, 2007).

In 1993, the International Seed Health Initiative-Vegetables (ISHI-Veg) was started to give stimulus to the vegetable seed industry to put more emphasis on seed health for quarantine pests and their impact on the international seed trade. Later, in the year 2000, the International Seed Federation (ISF) took over the secretariat and financial administration of ISHI-Veg. Nevertheless, in order to remain flexible and efficient, ISHI-Veg has maintained a special structure within ISF with separate funding by the participating countries. Further, ISF started two more ISHIs, viz. ISHI for herbage crops in 1997 and ISHI for field crops in 1999 (ISF 2022).

In 1995, during the second Seed Health Symposium of Plant Disease Committee, ISHI-Veg and ISTA jointly produced the Guidelines for Comparative Testing Methods for Detection of Seed-borne Pathogens (Sheppard and Wesseling 1998). These generalized test methods were particularly beneficial to the seed companies to make risk analysis specific to the conditions under which they operate, taking also other factors such as resistance in their varieties, seed production region, and the region where the seeds will be sold into consideration.

3.1 Advancements in Seed Health Testing Methodologies

Since the establishment of comparative seed health testing programme by the Plant Disease Committee of ISTA in 1957, seed health testing has undergone many changes. The seed health testing was primarily focused on the detection of seed-borne fungi and mostly relied upon incubation methods, morphological identification, or grow-out tests for the detection of these pathogens on seed. Presently, due to advances in technology, the seed-borne pathogens are detected both by conventional methods and using immunodiagnostic and molecular methods.

3.1.1 Conventional Methods

Direct Examination

This refers to the detection of such seed-borne pathogens which cause discoloration of the seed or cause change in the shape and size of the seed and hence are visibly

detectable. Visual examination also helps for detecting fungal structures present on or with seed, such as the sclerotia, galls, smut balls, discolouration, malformation, resting hypha, fruiting bodies of fungi (oospores, pycnidia, perithecia), and bacterial masses (Rao and Bramel 2000). Some examples of visibly detectable diseases are maize seeds infected with *Nigrospora*, which have white streaks with black spore masses near the tips; sorghum seeds infected with *Acremonium* wilt which are completely deformed; and soybean seeds infected with *Cercospora kikuchii* showing symptoms of purple seed stain. The fungus *Claviceps*, the cause of ergot of sorghum and pearl millet, often is mixed with seed as sclerotia. Yellow ear rot or tundu disease-infected wheat seeds can also be detected by visual inspection of black galls caused by the nematode *Anguina tritici* (Agarwal and Sinclair 1997) (Plate 1).

Seed Washing Test

The washing test is helpful for detecting surface-borne fungal spores causing smuts, bunts, rusts, downy mildews, powdery mildews, etc. and of bacterial crusts on seed surface (Maddox 1998). Spores of fungi or bacterial cells are washed from seeds with water, and then the suspension is centrifuged. The supernatant is discarded, and the pellet is re-suspended in sterile distilled water. This spore suspension is then examined under the microscope for the presence of fungal spores. The spore load per seed can be estimated using a haemocytometer.

NaOH Seed Soak Method

This method is used for the easy detection of *Tilletia indica* and *T. barclayana*, causing Karnal bunt in wheat and paddy kernel smut/bunt, respectively (Agarwal and Srivastava 1981). In this method, a sample of minimum 2000 seeds is soaked in sodium hydroxide solution (NaOH 2%) for 20 h at 20 °C. The infected seeds exhibit loose spores of shiny jet black in colour of the fungus at the infected portions in contrast to the pale yellow healthy seed. Upon rupturing the black seeds in a drop of water, a mass of teliospores is released (Plate 2). The sodium hydroxide treatment increases the colour contrast between diseased and healthy wheat and paddy seeds (Agarwal and Mathur 1992), making the detection easier.

Embryo Count Method

This technique was developed exclusively for the detection of loose smut fungi (*Ustilago tritici*) in wheat (Rennie 1982). The fungal mycelium is localized in the embryo of the seed. To separate the intact embryo, from the rest of the seed, for microscopic observation is not possible. Therefore, the embryos are released from the wheat seeds by soaking in a solution of NaOH (5%) + trypan blue (0.03%) for 12 h. The embryos thus released are processed, dehydrated with sprit, and cleared in a solution of glycerine + lactic acid (1:1) and examined under stereo binocular microscope. The test is performed with a sample size of minimum 2000 seeds (Cappelli and Covarelli 2005) (Plate 3).

Plate 1 (**a**) Karnal bunt (*Nevossia indica*)-infected wheat seeds. (**b**) Nematode galls (*Anguina tritici*) mixed with wheat seed. (**c**) Seeds infected with paddy bunt (*Tilletia barclayana*). (**d**) Common bunt-infected wheat seed (*Tilletia laevis*). (**e**) Soybean mosaic virus (SMV) seed discolouration. (**f**) Purple seed stain (*Cercospora kikuchii*) of soybean

Incubation Test

The incubation test is the most common and widely used method for detecting a large number of seed-borne pathogens. In this, the seeds are incubated on a substrate, under specific environmental conditions for specified times to allow pathogens to grow on the seed. At the end of the incubation period, seeds are examined for the fungal growth of the pathogen on each seed using a stereomicroscope or compound

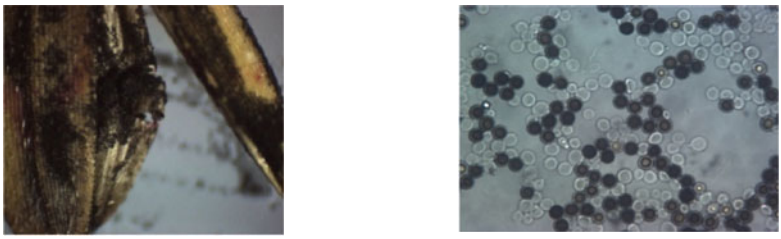

Plate 2 Detection of rice bunt by NaOH seed soak method. (**a**) Paddy seed infected with bunt. (**b**) Teliospores of *Tilletia barclayana*

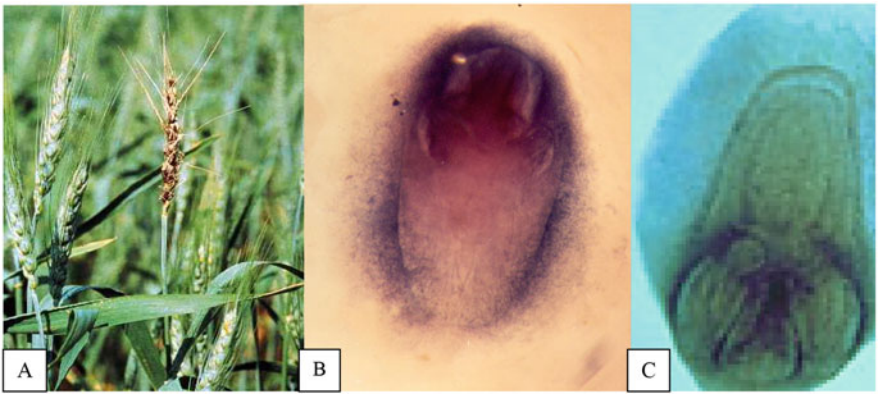

Plate 3 (**a**) Loose smut infected ear of wheat. (**b**) Wheat embryo showing loose smut infection by embryo count method. (**c**) Healthy non-infected embryo

microscope. Fungi are identified on the basis of their morphological characters, such as growth of the mycelium its septation, size and shape of the fruiting bodies, spores, their size and shapes, arrangement of conidia on conidiophores, etc. (Warham et al. 1990). Standard blotter, 2,4-D blotter, deep freezing blotter, and agar plate methods are the commonly used incubation methods for the detection of various seed-borne pathogens (Rao and Bramel 2000; Tsedaley 2015).

Doyer, in 1938, developed the standard blotter method, the most widely practised seed health testing incubation method, which was later included in the International Seed Testing Application Rules of 1966. Many laboratories still use this method as the first screening test for health condition of a seed lot. The blotter method is widely used for detecting fungi which are able to produce mycelial growth and fruiting structures under the incubation conditions (Plate 4). The method is good in testing seeds for fungi such as *Alternaria*, *Ascochyta*, *Bipolaris*, *Botryodiplodia*, *Botrytis*, *Cercospora*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Fusarium*, *Macrophomina*, *Myrothecium*, *Phoma*, *Phomopsis*, *Rhizoctonia*, *Sclerotinia*, etc.

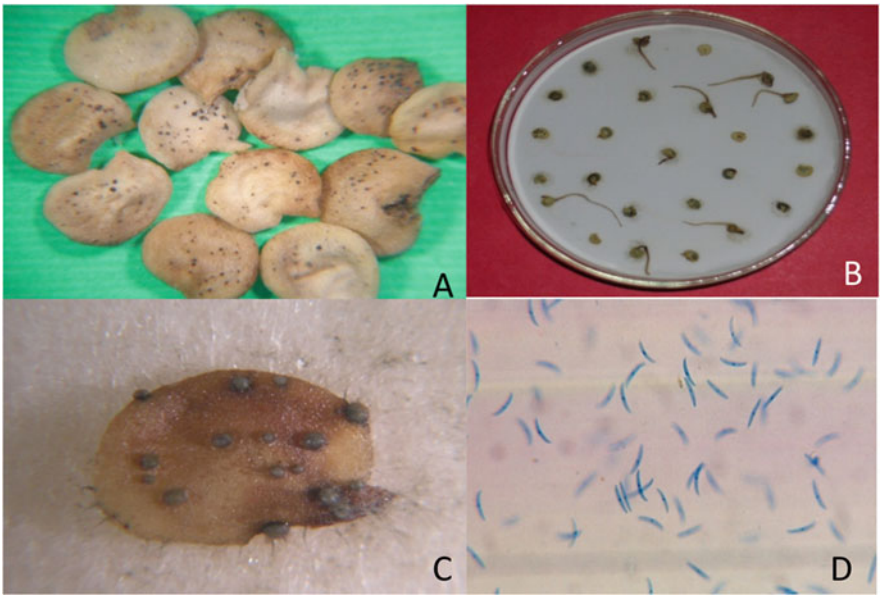

Plate 4 Blotter method for the detection of chilli anthracnose pathogen. (**a**) Infected seeds showing acervuli of the fungus. (**b**) Blotter test showing the growth of the fungus on seed surface. (**c**) Enlarged view of a single seed showing acervuli on seed surface. (**d**) Spores of *Colletotrichum capsici*

All kinds of cereals, vegetables, legumes, ornamentals, and forest seeds are tested by this method.

For routine seed examination, usually a seed sample of 400 seeds is used. In the standard blotter method, until and unless not specified otherwise, seeds are placed in 9 mm Petri dishes containing three-layered water-soaked blotter sheets as substrate (water-holding capacity of 40 cc). Seeds, sterilized with NaOCl solution (1.0%) and subsequently washed at least three times with sterilized water, are placed at 25 seeds/plate in small seeds and 10 seeds/plate in large seeds. These plates are incubated for 7 days at 20 ± 2 °C, under white fluorescent light and alternate cycle of 12 h light and 12 h darkness examined for the growth of the fungi under stereo binocular microscope. The major limitation of this method is that certain crop seeds germinate quite fast and obstruct the observations. To overcome this problem, the 2,4-D blotter method is used where the blotters, instead of soaking in ordinary water, are treated with a solution of 200 ppm of 2,4-D solution, as it either kills the seed or retards the seed germination and thus facilitates the easy observation of the seed samples. The rest of the procedure, incubation conditions, and period for incubation are the same as in the standard blotter method. The method was first used by Hagborg et al. (1950) for testing bean seeds for the presence of *Colletotrichum lindemuthianum*. However, this method too stances certain limitations because the 2,4-D reduces the recovery of certain fungi, and in that case, the deep freezing blotter method (Limonard 1966)

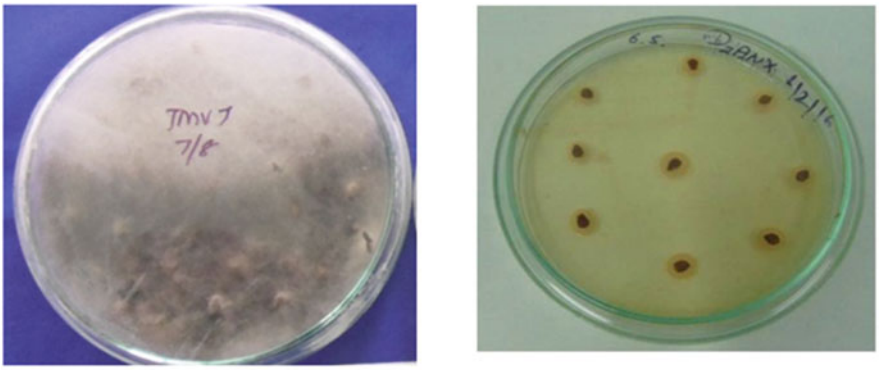

Plate 5 Agar plate method for the detection of seed-borne pathogens of sesame and tomato. (**a**) Sesame seeds showing *Macrophomina phaseolina* growth on PDA medium. (**b**) Tomato seeds showing *Clavibacter michiganensis* subsp. *michiganensis* growth on D2ANX medium

may be used. In this method, after plating the seed, as mentioned in the blotter method, the Petri dishes are incubated initially for 24 h at 20 ± °C and then transferred to a freezer at −20 °C for 24 h followed by a 5-day incubation at 20 ± 2 °C under white fluorescent light in alternate cycle of 12 h light and 12 h darkness.

In the agar plate method, the substrate used is the culture medium. Commonly, used media are either potato dextrose agar or Czapek's Dox agar or malt agar on which most of the fungi display their growth. Sterilized culture medium is poured in sterilized Petri plates of 9 mm diameter at 15 ml/plate. After solidification of the medium, seeds are placed in culture plates in the same way as in the blotter test. These plates are incubated under similar conditions as in the standard blotter test for the same period of time. However, certain pathogen requires selective or semi-selective media for their recovery or growth. For example, *Clavibacter michiganensis* subsp. *michiganensis* gives better recovery on D2ANX medium (Tripathi et al. 2018) (Plate 5).

Seedling Grow-Out Test

Certain seed-borne pathogens exhibit characteristic symptoms on developing seedlings, and thus, seedling grow-out test can be used as a direct method to assess the seed-borne pathogens on their living host and their transmission through seed. Under controlled greenhouse conditions, seedling symptom test reveals seed viability and helps detect the presence or absence of seed-borne pathogens on host plants. To perform this assay, seed samples are planted under controlled greenhouse conditions, conducive to disease development, and seedlings thus raised are examined for the appearance of symptoms (Plate 6). Seedling grow-out test is one of the most applicable and widely used assays for the detection of seed-borne pathogens in the living host (Lee et al. 1990; Yang et al. 1997). However, this test has certain

Plate 6 Grow-out test for the detection of urdbean leaf crinkle disease. (**a**) Naturally infected plant. (**b**) Screening of plants for seed transmission studies. (**c**) Plants exhibiting infection on exposure from nethouse

limitations as it may not always reveal the infection on the seedlings or the symptoms produced by certain seed-borne pathogens are not very distinct and conspicuous. In such cases, pathogens need to be isolated from suspected seedlings for confirmation. These additional steps further increase the time required to complete the seedling grow-out assay. Another limitation is that such test requires a large sample size to statistically confirm the infection percentage in seed. Besides, the test requires controlled conditions for the growth of seedlings and expression of symptoms failing, of which the symptoms are either obscure or ambiguous.

Conventional methods for seed health testing are mostly based on visual symptoms, culturing, and laboratory identification of the pathogens. These methods are performed at different levels (multi-stage) and time- and labour-intensive and in addition require extensive taxonomic expertise. They are not reliable at times and are difficult to apply in those cases where the symptoms are ambiguous or not expressed. Thus, within the last 25 years, advanced techniques for the accurate and feasible detection of the many seed-borne pathogens are developed. These include various immunoassays and nucleic acid-based techniques.

3.1.2 Immunoassay Methods

These methods are usually applied to detect many bacterial and viral pathogens. Among immunoassays, enzyme-linked immunosorbent assay (ELISA) is widely used to detect seed-derived fungi, bacteria, and viruses. Chang and Yu (1997) used DAS-ELISA for the detection of moulds, viz. *Aspergillus parasiticus*, *Penicillium citrinum*, and *Fusarium oxysporum*, in rice and corn seeds. The seed immunoblot binding assay (SIBA) has been an effective method in detecting *Tilletia indica*, in wheat seed. Immunoassays are popularly used in detecting mycotoxins produced by fungi such as *Aspergillus*, *Claviceps*, and *Fusarium* spp. The consistent use of ELISA for the detection of *Erwinia stewartii* in corn seeds has been demonstrated (Lamka et al. 1991). Detection and identification of *Xanthomonas oryzae* pv. *oryzae* in rice seed, using pathovar-specific monoclonal antibodies, could be performed using ELISA (Gnanamanickam et al. 1994). Likewise, ELISA has been an effective method for the detection of multiple viruses present in seeds (Fegla et al. 2000; Forster et al. 2001; Gillaspie Jr et al. 2001; Faris-Mukhayyish and Makkouk 2008; Ojuederie et al. 2009; Chalam et al. 2017; Torre et al. 2019).

The dot-immunobinding assay (DIBA) or dot-ELISA is similar to ELISA, except replacing the microtiter plate with a nitrocellulose or nylon-based membrane. The cut surface of the pre-soaked test seed is brought in-tuned with the membrane. The free protein binding sites present within the membranes are blocked using bovine serum albumin (BSA) or non-fat powdered milk, followed by the application of virus-specific antisera. The positive reaction is indicated with coloured dots. The presence of barley stripe mosaic virus and bean common mosaic virus in a single seed of French bean could be detected by the DIBA technique (Lange and Heide 1986). DIBA is optimized and used successfully for the rapid detection of 15 known soybean viruses as well (Ali 2017).

Flow cytometry (FCM) is a technique that enables fast multi-parameter analysis and quantification of the inoculum such as bacterial cells. The analysis is based on size and granularity and may be based on fluorescence after staining with a fluorescent dye. FCM has already been used in combination with antibody staining (immuno-FCM) to detect *Clavibacter michiganensis* subsp. *michiganensis* in tomato seed extracts and *Xanthomonas campestris* pv. *campestris* in cabbage seed extracts (Chitarra et al. 2002, 2006).

Immunoassays are suitable for the detection of seed-borne bacteria and viruses; however, the lack of species-specific antibodies remains a major constraint of their use for the detection of seed-borne fungal pathogens. Additionally, serology-based assays can also detect non-viable propagules which can lead to ambiguity in results (Mancini et al. 2016). Furthermore, these methods cannot detect all strains of pathogens and thus are limited in their applicability.

3.1.3 Molecular/Nucleic Acid-Based Diagnostic Methods

The detection, quantification, and characterization of seed-borne pathogens using multitude molecular marker are steadily increasing a routine practice for seed health testing. These molecular methods are now available for the detection of a number of seed-borne pathogens. In contrast to conventional seed health tests, DNA-based molecular techniques often have the advantage of being specific to the species level, sensitive, and rapid with the potential of being automated. Rapid detection of a specific pathogen in host tissue itself may be achieved using polymerase chain reaction (PCR). PCR-based assays have high sensitivity and specificity and often require as little as 24 or 48 h to complete. They are applicable to a wide range of pathogens and can be used to separate closely related species (see Chap. 15 for more).

Molecular methods have also been worked out for a number of seed-borne fungal pathogens of significance such as *C. purpurea* (ergot) (Correia et al. 2003), *Microdochium nivale* (foot rot) (Scherm et al. 2013), and *T. tritici* (bunt) (Majumder et al. 2013; Anil et al. 2008) in wheat and *Plenodomus lingam* (black leg) in cabbage (Mancini et al. 2016). PCR-based assay using internal transcribed spacer (ITS) primers specific to the regions of the ribosomal repeat (rDNA) was developed for the identification of the three *Alternaria* species on carrot seeds. The primers were highly specific, sensitive, and capable of differentiating the fungal pathogens (Konstantinova et al. 2002). Black spot disease of crucifers caused by *Alternaria*

sp. is an important seed-borne disease. PCR-based diagnostic procedure involving the use of specific primers designed from DNA sequence in the ITS region of nuclear rDNA was employed to detect and differentiate *Alternaria brassicae*, *A. brassicicola*, and *A. japonica*, causal agents of black spot of crucifers. These pathogens were detected in the DNA extracted from seed macerates (Iacomi-Vasilescu et al. 2002).

By employing two different sets of primers, the conventional and real-time PCR, *A. brassicae* was specifically detected in the DNA extracted from seed. The presence of seed-borne pathogens such as *A. brassicicola* and *A. japonica* in radish; *A. alternata* in radish and cabbage; *Stemphylium botryosum*, *Penicillium* sp., and *Aspergillus* sp. in cabbage; and *Verticillium* sp. in tomato seeds was detected by the quantitative real-time PCR (Guillemette et al. 2004).

The BIO-PCR technique involves a combination of biological and enzymatic amplification of DNA sequences of target bacteria. Using this method, it is possible to detect *P. syringae* pv. *phaseolicola*, even if only 1 seed in a lot of 400 to 600 seeds is infected (Mosqueda-Cano and Herrera-Estrella 1997).

Incorporation of an immunological step before PCR assay significantly improves the sensitivity of detection of target bacteria present in seeds. Immunomagnetic separation (IMS) was performed before PCR assay to concentrate *A. avenae* subsp. *citrulli* present in watermelon seeds. A significant increase in sensitivity (100-fold) of detection by IMS-PCR was observed in comparison to an on-the-spot PCR assay, the detection limit being 10 CFU/ml. As low as 0.1% of seed infection was revealed by IMS-PCR assay (Walcott and Gitaitis 2000).

However, these methods are applied to detect viruses in seeds in mere some cases. In the case of RNA plant viruses, reverse transcriptase is employed to provide a complementary cDNA before PCR assay for the detection of the virus concerned within the seeds. *Clavibacter michiganensis* subsp. *michiganensis* in tomato seed extracts (Tripathi et al. 2022) and pea seed-borne mosaic virus (Kohnen et al. 1992) and cucumber mosaic virus in lupin seeds (Wylie et al. 1993) were detected by RT-PCR assay. Similarly, CABMV may be detected in samples consisting of 1 infected and 99 healthy leaves, indicating the sensitivity of the RT-PCR assay (Gillaspie Jr et al. 2001). RT-PCR was found rapid and sensitive in detecting viruses in seeds of vegetables (Gumus and Paylan 2013). Nonetheless, for the detection of several seed-borne pathogens, with real-time PCR, it's crucial to choose the appropriate target DNA fragments to design the primers and probes with adequate specificity and sensitivity and comparable amplification (Mancini et al. 2016).

A sensitive multiplex RT-PCR-based method could simultaneously assay the presence of five seed-borne legume viruses such as alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), cucumber mosaic virus (CMV), and subterranean clover mottle virus (SCMoV) (Bariana et al. 1994). Primers are so designed that the size of RT-PCR product was indicative of the virus amplified and the sequence of more than one stain of virus was available.

Recently, a simple, rapid, and cost-effective method for nucleic acid amplification termed loop-mediated isothermal amplification (LAMP) has been developed for the detection of various plant pathogens. A sensitive reverse transcription loop-mediated

isothermal amplification (RT-LAMP) method is developed for the rapid detection of BSMV isolates. The RT-LAMP assay can be used for the routine diagnostics of BSMV in seed and plant material (Zarzyńska-Nowak et al. 2018).

4 Management of Seed-Borne Diseases

4.1 Management of Seed-Borne Diseases Through Crop Production Practices

4.1.1 Identification of Disease-Free Areas for Healthy Seed Production

An important consideration for seed production is to select the site and the field where the climatic conditions are favourable for the plant to grow, flower, and set seed. Besides, it is also important to know about the past history of the field such as previous crops cultivated, weed populations, predominant diseases, and other intrinsic factors for successful disease-free seed production. For example, chickpeas should not be planted on the land on which lucerne was previously grown, as *Phytophthora* root rot affects both the crops (Ogle and Dale 1997).

Some plant pathogens are more pronounced in certain geographical areas, and hence, to escape the diseases, such areas need to be avoided for seed production. In general, most fungal and bacterial diseases are more pronounced in wet areas than in dry areas, e.g. ergot and smut of pearl millet. Healthy seed production is, therefore, recommended in those areas and seasons which are not predisposed for disease development. In addition, the same crop must not be cultivated in the same field year after year.

4.1.2 Time of Sowing

Altering the sowing or planting date can help to reduce the disease outbreaks by avoiding the weather conditions favourable for pathogens to grow, multiply, and infect the crop. Prolonged wet weather favours the occurrence of many diseases, e.g. downy mildew requires prolonged high moisture to cause infection. Chickpea (*Cicer arietinum*) root rot caused by *Rhizoctonia* spp. intensifies if the crop is sown immediately after rainfall. Early maturing varieties of wheat and pea are able to escape infection by *Puccinia graminis tritici* and *Erysiphe polygoni*, respectively. Similarly, in pearl millet, early sowing reduces the incidence of ergot disease (Gupta and Kumar 2020). Early planting also helps to avoid a pronounced bacterial black rot disease in crucifers (*Xanthomonas campestris*) because the environmental conditions are usually dry and not conducive for the development and spread of the pathogen.

4.1.3 Cultural Practices

The aim of the good cultural practices is to create favourable environmental conditions for crop development, promote good plant health, and limit the spread of plant pathogens, thereby minimizing the disease outbreaks. Some common practices include tillage, removal, and destruction of diseased crop residues or

debris, eradication of alternate and collateral hosts, cultivation of non-host crops, selection of disease-free seeds, maintenance of appropriate isolation distances, timely rouging of infected plants and weeds, adequate irrigation, and balanced fertilization (Gupta and Kumar 2020).

A minimum isolation distance between seed production plots and commercial plots must be maintained to reduce the incidence of loose smut in wheat and barley seed crops. However, the distance recommended between seed plots and commercial plots may vary from one region to another depending upon the prevailing weather conditions. In countries, such as Germany and the United Kingdom, the distance between seed production plot and commercial cultivation plots for wheat and barley is maintained 50 m, whereas in the Netherlands, for barley, a minimum distance of 100 m between seed production plots and commercial field is secured. However, in India, for loose smut disease, an isolation distance of at least 150 m is recommended between seed plots and commercial field in the case of wheat, barley, oat, and rye (IMSCS 2013).

There are a number of the cultural practices which influence the incidence and severity of diseases such as spacing between the rows and between the plants in a row, time and methods of sowing/planting, depth of sowing, time and number of irrigation, quantity and composition of fertilizers/organic manures, cropping patterns, etc. In the nursery, overcrowding can lead to seedling damping off resulting in seedling's death. The incidence of bunt and smut in wheat is higher in deeply sown crops. Deep ploughing of the soils can effectively reduce the inoculum of *Phytophthora infestans*. Sowing of trap crops stimulates the dormant pathogen, and thus the host crop gets protected from pathogen attacks. Similarly, mixed cropping may reduce or increase the disease incidences. For instance, when soybean and maize are grown together, soybean rust (*Phakopsora pachyrhizi*) is more pronounced, and when maize and cowpea are grown together, anthracnose (*Colletotrichum lindemuthianum*) is more severe on the cowpea than when a single crop is grown (Ogle and Dale 1997). Similarly, less incidence of *Macrophomina* stem and root rot was observed in sesame, grown as a mixed crop or intercropped with green gram (Rajpurohit 2002). Crop rotation and burning of stubble also helps to reduce the build-up of inoculum affecting seed setting. Good control of *Cephalosporium gramineum* and *Pseudocercosporella* spp. in cereal crops has been obtained following stubble burning (Ogle and Dale 1997). Crop rotation also helps to reduce the build-up of pathogens causing seed rot (Francl et al. 1988).

The most effective disease control strategy is to use, as far as possible, the disease-resistant or disease-tolerant varieties and disease-free seeds for cultivation and adopt a cropping pattern which does not aggravate a disease.

4.2 Seed Certification for the Management of Seed-Borne Diseases

Seed certification is a regulatory management practice for disease-free seed production and to reduce the seed-borne infection. Seed certification ensures that the seed

has certain set standards and the quality and history of each seed lot are evident and may be traced. Seed certification procedures are established to maintain the standards of purity and permissible level of infection in both field and laboratory testing. Certain legislation and standard have been recommended by the seed certification board/committee for seed testing including seed health; however, any organization/country/state may enforce even higher voluntary standards (HVS) for varietal and physical purity, for weed seed contents, and for seed health in a seed lot.

Seed certification includes testing of seed lots before sowing as well as after harvesting and crop inspection in the field in compliance with standards set forth for isolation and freedom from diseases. Implementation of seed certification procedures helps to regulate and control the distribution and spread of certain seed-borne pathogens to uninfected newer areas. Field inspection during seed production helps to reject the seed lots with high incidence of seed-transmitted pathogens there in the field itself. Nevertheless, certain seed-borne pathogens are carried through seed asymptomatically. For that matter, seed samples are tested under laboratory conditions for notified seed-borne diseases under seed certification programme, and those seed lots exhibiting higher level of seed infection than prescribed are rejected. Despite the fact that seed certification standards (tolerance limit) for seed-borne diseases are difficult to establish in different categories of seed (basic, foundation, or certified seed), inoculum threshold has been established with supportive correlation data for a few seed-borne pathogens such as *Phoma lingam*, *Xanthomonas campestris* pv. *campestris*, *Pseudomonas syringae* pv. *phaseolicola*, and *Diaporthe phaseolorum* var. *sojae* (Kuan 1988).

There are many examples of successful seed health management through seed certification. In Australia, certification of bean seed production (*Phaseolus vulgaris* L.) began in the 1930s in New South Wales and Victoria as a measure against the devastating diseases bacterial blight and anthracnose (Persley et al. 2010). Successful management of barley stripe mosaic virus (BSMV) following seed certification prevented yield losses in Montana (Carroll 1983) and North Dakota, USA. At both the places, the programme followed field inspection, supplemented with serological assays (Carroll et al. 1979) of the foundation seed lot. In North Dakota, no specific field inspection is done, but the samples of foundation seed, having 1000 seeds in each seed lot, are assayed by ELISA. In either case, only the virus-free seeds are certified. Similarly, schemes based on rigorous field inspections were a complete success for the management of seed-borne fungal diseases (Gabrielson 1988). A standard of no infection of *Plenodomus lingam* in brassica, *Septoria apiicola* and *Phoma betae* on celery, *Colletotrichum lindemuthianum* and *Pseudomonas syringae* pv. *phaseolicola* on beans, and *Ascochyta fabae* in broad bean for seed multiplication is proposed (Hewett 1979a, b). Besides, inoculum thresholds for seed-borne fungi (Gabrielson 1988), bacteria (Schaad 1988), and viruses (Stace-Smith and Hamilton 1988) are used as part of disease management. In India, Karnal bunt of wheat was the first disease for which tolerance limit was fixed for seed-borne inoculum as early as in 1970, and strict field inspection, followed by seed testing, could effectively control this disease.

In India, in 1971, the seed certification standards were formulated and published in the manual of 'Indian Minimum Seed Certification Standards', which contained general seed certification standards applicable to all major crops. These minimum seed certification standards are further revised and upgraded in 1988 and in 2013 based on information generated through scientific studies.

4.2.1 Designated Seed-Borne Diseases

Diseases specified by national regulatory authority for the certification of seeds and for which certification standards must always be complied with are known as designated diseases. These diseases would cause contamination if present in the seed field or within the specified isolation distance. In order to produce disease-free seeds, some diseases are designated, and standards have been fixed for those diseases in concerned crops in India. The permissible limits of seed-borne designated diseases of important agricultural and horticultural seed crops are listed in Table 1 and exemplified in Plate 7. During field inspection, the guidelines given in the Indian Minimum Seed Certification Standards should be carefully followed by checking the symptoms of these designated diseases at different plant stages. Simultaneously, compliance for seed health testing under laboratory conditions as per the set standards should be followed.

Also, use of tissue culture and micro-propagation techniques helps in the multiplication of pathogen-free planting stock which can be grown in greenhouse to create a barrier for disease incidents and their spread (IMSCS 2013).

4.3 Management of Seed-Borne Diseases Through Quarantine Regulations

Plant quarantine is a legislative procedure to exclude the plant pathogens from invading into an area where they do not exist, by monitoring the import and export of plant, seeds, grafts, planting material, or equipment to prevent the spread of diseases and pests through these sources.

Plant quarantine may, therefore, be defined as 'Rules and Regulations' proclaimed by the government(s) to regulate the introduction of plants, planting materials, plant products, soil, living organisms, etc. from one region to another with a view to prevent unintended introduction of exotic pests, weeds, and pathogens harmful to the agriculture or to the environment of a region or country and, in case introduced, prevent their establishment and further spread without adversely affecting the trade (Gupta and Khetarpal 2004). Quarantine, thus, aims to prevent the introduction of dangerous pathogens, but not to stop the movement of other biological material.

Initially, it was France where a Quarantine Act was enacted in 1660 for the eradication of barberry plants. Subsequently, Germany in 1873, Britain in 1866, and the United States in 1912 passed Acts and legislations to prohibit the entry of plants or planting material carrying pest and diseases harmful to agriculture.

Table 1 (A, B, and C) Designated seed-borne diseases and permissible limits for important agricultural and horticultural seed crops in India (IMSCS 2013)

A. Designated diseases and their maximum permissible limits in seed

Crop	Disease/causal organism	Seed standards (%)	
		Foundation	Certified
Wheat and hybrids	Karnal bunt (*Tilletia tritici*)	0.05	0.25
Paddy and hybrids	Bunt (*Tilletia barclayana)*	0.10	0.50
Sorghum and hybrids	Ergot (*Claviceps sorghi*) (teleomorph) (*Sphacelia sorghi*) (anamorph) Sclerotia, seed entirely or partially modified as sclerotia, broken sclerotia, or ergotted seed	0.02	0.04
Pearl millet and hybrids	Ergot (*Claviceps sorghi*) (teleomorph) (*Sphacelia sorghi*) (anamorph) Sclerotia, seed entirely or partially modified as sclerotia, broken sclerotia, or ergotted seed	0.02	0.04
Triticale	Karnal bunt (*T. indica* = *Tilletia tritici*)	0.05	0.25
Forage sorghum including Sudan grass	Ergot (*Claviceps* spp. = *Sphacelia sorghi*) Sclerotia, seed entirely or partially modified as sclerotia, broken sclerotia, or ergotted seed	0.02	0.04
Sweet potato	Storage rots Scurf (*Monilochaetes infuscans*) Wilt (*Fusarium oxysporum* f.sp. *batatas*) Block rot (*Ceratostomella fimbriata*) Internal cork Nematode	None None None None 5.0 None	None 1.0 1.0 1.0 5.0 1.0
Ginger	Dry rot	1.0	5.0
	Phyllosticta	5.0	10.0
Turmeric	Dry rot	1.0	5.0

B. Designated diseases and their maximum permissible limits in field

Crop group	Disease/causal organism	Field standards (%)	
		Foundation	Certified
Cereals			
Wheat and hybrids	Loose smut (*Ustilago tritici*)	0.10	0.50
Sorghum and hybrids	Kernel smut (*Sphacelotheca sorghi* = *Sporisorium sorghi*) Head smut (*Sphacelotheca reiliana* = *Sporisorium reiliana*)	0.050	0.10
Barley and hybrids	Loose smut (*Ustilago nuda* = *Ustilago segetum* var. *nuda*)	0.10	0.50
Oat	Loose smut (*Ustilago nuda* = *Ustilago segetum* var. *avenae*)	0.10	0.50
Pearl millet and hybrids	Grain smut (*Tolyposporium penicillariae*) Downy mildew/green ear (*Sclerospora graminicola*) Ergot (*Claviceps microcephala* = *C. fusiformis*)	0.050 0.050 0.020	0.10 0.10 0.040
Triticale	Ergot (*Claviceps purpurea*)	0.020	0.040

(continued)

Table 1 (continued)

B. Designated diseases and their maximum permissible limits in field			
		Field standards (%)	
Crop group	Disease/causal organism	Foundation	Certified
Oilseeds			
Sesame	Leaf spot (*Cercospora sesami*)	0.50	1.0
Sunflower and hybrids	Downy mildew (*Plasmopara halstedii*)	0.050	0.50
Pulses			
Green gram	Halo blight (*Pseudomonas phaseoli* = *P. savastanoi* pv. *phaseolicola*)	0.10	0.20
Bean legume vegetable			
Cowpea	• Ashy stem (*Macrophomina phaseolina*) • Anthracnose (*Colletotrichum lindemuthianum*) • Blight (*Ascochyta* spp.) (for hill areas only) • Cowpea mosaic	0.10	0.50
French and Indian bean	• Bacterial blight (*Xanthomonas* spp.) • Anthracnose (*Colletotrichum lindemuthianum*) • Blight (*Ascochyta* spp.) (for hill areas only) • Bean mosaic	0.10	0.20
Cluster bean	• Bacterial blight (*Xanthomonas campestris* pv. *cyamopsidis*) • Anthracnose (*Colletotrichum lindemuthianum*) • Blight (*Ascochyta* spp.) (for hill areas only)	0.10	0.20
Cucurbits			
Musk melon and hybrids	• Cucumber mosaic virus (CMV)	0.10	0.20
Summer squash	• CMV • Watermelon mosaic virus (WMV)	0.10	0.50
Solanaceous vegetables			
Eggplant	• *Phomopsis* blight (*Phomopsis vexans*)	0.10	0.20
Eggplant hybrids		0.10	0.50
Chilli	• Leaf blight (*Alternaria solani*) • Anthracnose (*Colletotrichum capsici*)	0.10	0.50
Tomato and hybrids	• Early blight (*Alternaria solani*) • Leaf spot (*Stemphylium solani*) • Tobacco mosaic virus	0.10	0.50
Leafy vegetables			
Celery	• Leaf blight (*Septoria apiicola*) • Root rot (*Phoma apiicola*)	0.10	0.50
Lettuce	• Lettuce mosaic virus	0.10	0.50
Parsley	• Leaf spot (*Septoria petroselini*)	0.10	0.50

(continued)

Table 1 (continued)

B. Designated diseases and their maximum permissible limits in field			
		Field standards (%)	
Crop group	Disease/causal organism	Foundation	Certified
Cole crops			
Cabbage, cauliflower, broccoli, turnip, radish	• Black leg (*Leptosphaeria maculans*) • Black rot (*Xanthomonas campestris* pv. *campestris*) • Soft rot (*Erwinia carotovora*)	0.10	0.50
Fibre crops			
Jute	• Jute chlorosis	1.00	2.00
Forage crops			
Forage sorghum including Sudan grass	Kernel smut (*S. sorghi*) Head smut (*S. reiliana*)	0.050	0.10
Others			
Sweet potato	• Black rot (*Ceratocystis fimbriata*)	None	None
Plant bed	• Wilt (*Fusarium oxysporum* f.sp. *batatas*) • Scurf (*M. infuscans*)	None None	None None
Seed bed	• Wilt (*Fusarium oxysporum*) • Mosaic	0.050	0.10
Taro (Arvi)	• *Phytophthora* rot (*Phytophthora colocasiae*) • Dasheen mosaic	None 0.50	None 0.10
Tapioca	• Mosaic	0.10	0.50
Flower crops			
Annual carnation	• Mosaic (streak mosaic virus)	0.10	0.20
Chrysanthemum spp.	Grey mould (*Botrytis cinerea*) • Blotch (*Septoria chrysanthemella*)	0.10	0.20
Marigold	Leaf spot (*Alternaria tagetica*) Flower bud rot (*A. alternata*) Collar rot (*Rhizoctonia solani*)	0.10	0.20
Ornamental sunflower (*Helianthus* spp.)	• Downy mildew (*P. halstedii*)	0.050	0.050
Petunia/hybrid	Leaf blotch (*Cercospora petuniae*) Leaf spot (*Ascochyta petuniae*) *Phyllosticta* leaf spot (*Phyllosticta petunia*) Leaf blight (*Alternaria alternata*) Crown rot (*Phytophthora parasitica*) Tobacco mosaic virus (TMV) Cucumber mosaic virus (CMV)	0.10	0.20
Snapdragon/hybrid	• Anthracnose (*Colletotrichum antirrhini* and *C. fuscum*) • Blight (*Phyllosticta antirrhini*)	0.10	0.20

C. Certification for micro-propagation/tissue culture recommended for disease-free propagation	
Crop	Diseases
Apple	Apple mosaic virus, apple chlorotic leaf spot virus
Bamboos	Bamboo mosaic virus (BaMV)

(continued)

Table 1 (continued)

C. Certification for micro-propagation/tissue culture recommended for disease-free propagation	
Crop	Diseases
Banana	Bunchy top virus, cucumber mosaic virus, banana bract mosaic virus, banana streak virus
Black pepper	CMV and *Badnavirus*
Citrus	Indian citrus ringspot virus (ICRSV), citrus tristeza virus (CTV), and citrus yellow mosaic virus (CYMV)
Potato	Potato virus A, potato virus S, potato virus M, potato virus Y, potato virus X, potato leafroll virus (PLRV), potato apical leaf curl virus (PALCV), potato spindle tuber viroid (PSTVd) Wart (*Synchytrium endobioticum*) Cyst-forming nematodes Brown rot (*Pseudomonas solanacearum*) Common scab (*Streptomyces scabies*)
Sugarcane	Sugarcane mosaic virus Yellow leaf and *Luteovirus* Grassy shoot (*Candidatus Phytoplasma sacchari*) Red rot (*Colletotrichum falcatum*) Smut (*Sporisorium scitamineum*)
Vanilla-tissue culture	Vanilla mosaic potyvirus Vanilla necrosis potyvirus Cymbidium mosaic potexvirus Odontoglossum ringspot tobamovirus Uncharacterized potyvirus/rhabdovirus

In India, the Destructive Insects and Pests Act, 1914, provides regulatory measures for the protection of plants and planting material. India has a well-established network to offer plant quarantine services at both national and state level. The Directorate of Plant Protection, Quarantine and Storage, Ministry of Agriculture and Farmers Welfare, Government of India, monitors the plant quarantine services at national level. Plant quarantine services are rendered through 73 plant quarantine stations in the country at different airports, seaports, and land frontiers with headquarter at Faridabad. In India, domestic quarantine under DIP act is in place to restrict the movement of invasive pests, viz. flute scale, San Jose scale, coffee berry borer, codling moth, banana bunchy top, mosaic viruses, potato cyst nematode, potato wart, and apple scab. Many plant pathogens, for instance, downy mildew (*Sclerospora graminicola*) spores and ergot (*Claviceps fusiformis*) sclerotia in pearl millet, contaminate the seeds during threshing and may disseminate the inoculum with the infected seed to other un-infested areas. The ICAR-National Bureau of Plant Genetic Resources (NBPGR) is the nodal agency authorized to undertake quarantine processing of all the plant genetic material including transgenics and research material in the country and for issuance of import permit under PQ order, 2003 (Regulations of Import into India). NBPGR has intercepted and detected several pathogens, not prevalent in India, in the imported plant

Plate 7 Important designated diseases in different crops

germplasm using conventional, serological, and molecular techniques (Chalam et al. 2017; Bhalla et al. 2018).

4.4 Management of Seed-Borne Diseases Through Seed Treatment

Seed treatment refers to an application of physical, chemical, biological, or organic matter(s) to the seed to provide protection against pests and pathogens to germinating seed and seedlings and to improve the establishment of healthy crops. Seed treatment improves the seed quality and manages the seed-borne pathogens. Seed treatment not only benefits in seed disinfestations by cleaning the spores, mycelia, or propagules of microorganisms on the seed surface but also supports seed disinfection by eliminating the pathogen that has penetrated deep into the living cells of seed (e.g. smut or bunt) and gives protection to germinating seedlings from soil-borne pathogens. Various methods are applied for seed treatment.

4.4.1 Physical Seed Treatment

Physical seed treatments consist of heat treatments, most common being hot water and hot air treatments. The oldest practice of physical seed treatments is hot water treatment, where the seeds are immersed in hot water at a precise temperature for a certain period. Hot water treatment was generally practised to sterilize contaminated cereal seeds (Gilbert et al. 2005), though Nega et al. (2003) reported the successful management of seed-borne pathogens, viz. *Alternaria* spp., *Phoma* spp., *Peronospora valerianellae*, *Septoria* spp., and *Xanthomonas* spp., in carrots, celery, parsley, and lettuce by hot water treatment. Koch et al. (2010) observed hot air treatment of carrot seeds as effective as chemical treatment for the management of seed-borne infection of *Alternaria dauci* and *A. radicina*.

Aerated steam treatment has been highly effective in multiple host pathogen systems (Forsberg et al. 2002; Tinivella et al. 2009; Schaerer 2012) and exhibited an effective management of seed-borne infection of *Septoria apiicola* in celery, *F. moniliforme* in sweet corn, *C. michiganensis* subsp. *michiganensis* in tomato, and *X. campestris* in cauliflower (Groot et al. 2006, 2008). Precisely managed aerated steam seed treatment kills the pathogen leaving seeds intact without adversely affecting the seed vigour and viability.

Electronic seed treatment is a new seed treatment method which may help to destroy the DNA of harmful organisms present on seed surface. However, there is a need for further investigation to reach an inference whether this method of seed treatments can be used to eradicate pathogens on seed surface (Schmitt et al. 2006).

4.4.2 Chemical Seed Treatment

Application of fungicidal seed treatments improves seed emergence and plant vigour and avoids the transmission of seed-borne pathogens from seed to seedlings. In addition, chemical seed treatments protect the emerging seed and seedlings from soil-borne pathogens as well. Two organic fungicides captan (dicarboximide fungicide) and concurrently thiram (dithiocarbamate), when introduced, were widely applied for seed treatment in various crops. Systemic fungicides, carboxin and thiabendazole, introduced in the early 1970s also got wide acceptability for seed treatment in a number of crops. Systemic fungicide is an important disease control strategy for several agricultural and horticultural crops worldwide. In India, benzimidazole fungicides are registered for use in 18 crops including rice, wheat, barley, peanuts, cotton, jute, mango, apples, grapes, beans, eggplants, cucurbits, peas, sugar beets, tapioca, and roses. Seed treatment with systemic fungicides is considered as an economically viable disease management strategy for several agricultural and horticultural crops worldwide (Bhushan et al. 2013; Lamichhane et al. 2020). Some other classes of fungicides, such as phenylpyrroles, phenylamides, strobilurins, and triazoles, are also used for the management of a number of seed-borne diseases through seed treatment (Zeun et al. 2013).

Fungicides introduced in recent past are the formulations of mixtures of multiple active ingredients with different mechanisms of action to enhance control of a variety of pathogens, e.g. strobilurins (e.g. azoxystrobin, pyraclostrobin, and trifloxystrobin), triazoles (e.g. difenoconazole, tebuconazole, and prothioconazole), pyrazole, and carboxamides (e.g. sedaxane). These systemic fungicides are used as seed dressing fungicides.

Seed treatments are commonly applied as seed dressing, seed coating, or seed pelleting before sowing (Pedrini et al. 2017). Seed dressing is the most common seed treatment method and involves dressing the seed with a dry or wet formulation of a fungicide. Seed dressing with fenfuram, triadimefon, triadimenol, tebuconazole, and hexaconazole enabled the effective control of wheat flag smut caused by *Urocystis agropyri* (Singh and Singh 2011; Shekhawat and Majumdar 2013; Kumar et al. 2019).

Seed coating and pelleting require a special binder to be used with the formulation to improve the adherence of the product to seed surface. Anwar and Shafi Bhat

(2005) evaluated several fungicides, isoprothiolane, tricyclazole, hexaconazole, and mancozeb, as seed coatings in two different doses and found isoprothiolane and tricyclazole to be the most effective in controlling the nursery blast disease. Seed pelleting requires specialized machinery and application techniques; in this case, fungicides can be segregated at different layers of the coating (Ahmed and Kumar 2020), to make it more effective.

4.4.3 Biological Seed Treatment

Application of beneficial antagonistic microbes to seed for managing seed- and soil-borne pathogens is a classical delivery system which reduces water pollution from chemicals, enriches soil microbiota, and is safe for the environment. Seed treatment with microbial antagonists protects seeds and seedlings from various pathogens. Majority of microbial antagonists are bacteria (84%) and fungi (16%). In the last decades, various bacterial biocontrol agents have been identified and used for the management of seed-borne diseases. Various bacteria which are mainly investigated and successfully commercialized as the biological control agents are *Pseudomonas fluorescens*, *Bacillus subtilis*, *Serratia marcescens*, *Streptomyces griseoviridis*, and *Burkholderia cepacia* (Singh 2014; Bisen et al. 2015; Keswani et al. 2016; Gouda et al. 2018). Species of *Pseudomonas* and *Bacillus* are the most commonly used bacterial biocontrol agents in controlling various phytopathogens including seed-borne pathogens (Abhilash et al. 2016; Bhat et al. 2019; Khan et al. 2020). Similarly, various fungi have also been studied for their effect as biocontrol agents on seed-borne pathogens. The important fungal biocontrol agents are the species of *Phomopsis*, *Ectomycorrhizae*, *Trichoderma*, *Cladosporium*, *Gliocladium*, etc. Among various fungal biocontrol agents, *Trichoderma* spp. are widely studied and globally used biopesticide (Singh 2006; Keswani et al. 2013; Bisen et al. 2016; Singh et al. 2016). *Trichoderma* spp. are potential plant symbionts and reported for their antagonistic activity against a wide range of seed- and soil-borne fungi. More than 60% of the global biopesticide market is based on *Trichoderma* formulation (Keswani et al. 2013).

Extracts from several plant species are also known to contain natural antimicrobial compounds which can be used for seed disinfection as an alternative to fungicidal treatments, singly or in combination (Begum et al. 2010). These extracts include essential oils, showing virtuous antifungal activities in in vitro trials using tea, clove, peppermint, rosemary, laurel, oregano, and thyme oils. Such oils have been reported to be active against pathogens like *Drechslera*, *Ascochyta*, and *Alternaria* spp. (Alice and Rao 1986; Riccioni et al. 2013). Of these, thyme oil which contains thymol and other antifungal compounds has shown greater efficacy in both in vitro and field tests against seed-borne fungi and bacteria (Van der Wolf et al. 2008). Other effective natural antifungal compounds have been extracted from plants that belong to the genus *Allium*, which produce various sulphur-containing compounds with antimicrobial effects (Nelson 2004; Lanzotti 2006).

5 Conclusions

Seed-borne pathogens pose a serious threat to crop establishment and yield. Seeds also serve as a way of dispersal and survival of plant pathogens. Therefore testing of seed lots for the presence of the pathogen is the most efficient way to avoid spread of diseases. Seed health testing and detection are the first line of approach in managing seed-borne diseases of plants. This can most effectively be accomplished by exclusion, using seed detection assays to identify contaminated seed lots that can be discarded or treated. In comparison to conventional and serological techniques, PCR assays have much higher sensitivity and specificity and often require very short time to detect the pathogens associated with seed. These are applicable to a wide range of pathogens and can be used to separate closely related species. Different modified PCR techniques including real-time PCR, BIO-PCR, IMS-PCR, RT-PCR, and IC-RT-PCR and LAMP assay hold great potential for enhancing pathogen detection in seeds, because it embodies some of the key characteristics, which include specificity, sensitivity, rapidity, ease of implementation and interpretation, and applicability (see chapter on Molecular Techniques for Testing Genetic Purity and Seed Health for more details).

Many techniques, measures, strategies, and procedures are applied in the management of seed-borne diseases in both field and horticultural crops. These techniques include (1) agronomic practices, viz. selection of disease-free areas for seed production, use of disease-resistant crop varieties and disease-free seeds for cultivation, adjustment of the time of sowing, removal and destruction of pathogen-infected crop residues, alternate and collateral weed hosts, timely rouging of designated disease-infected plant and/or plant parts in the seed production plot, proper isolation distance, crop rotation, and balanced nutrient management, (2) seed certification, (3) quarantine regulations, and (4) seed treatment methods, viz. physical, chemical, and biological methods. The treatments of seeds with fungicides or biocontrol agents represent good methods for their protection, disinfestation, or disinfection from seed-borne pests and pathogens. The success of such treatments relies upon the pathogen localization on the seed level and can provide improved crop stand in the field and increased yields.

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