

A mini-review on the development and emerging perspectives of seed pathology

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ABSTRACT

Seeds are the means of propagating about 90% of all food crops in the world, and they significantly influence yield potential in crop plants. Moreover, seeds are adversely affected by a number of factors such as postharvest and storage disease pathogens and unfavourable environmental conditions. Seed-borne pathogens represent a major threat to crop establishment and yield. Exposure of seeds to disease pathogens and other adverse conditions disrupts their normal physiology and metabolism which ultimately affects productivity. It is therefore imperative to promptly diagnose, treat, prevent and control seed related diseases. As with every other sphere of scientific studies, seed pathology has evolved tremendously over time; hence in this paper the development and emerging perspectives of this all important discipline was reviewed. Pathogen exclusion by detection and elimination of infested seed lots is required for disease management but traditional and conventional measures such as visual examination, use of media culture and serology are inadequate to detect pathogens associated with seed. In contrast, Nucleic acid-based molecular tools, such as the polymerase chain reaction (PCR) have emerged as faster and more reliable means for detecting and quantifying pathogens in seeds than conventional techniques.

Keywords: Seed pathology, diagnosis and control, polymerase chain reaction.

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INTRODUCTION

Seeds are indispensably vital to human nature and existence. They are an essential component of the world market, and are distributed globally (Ryan, 2009). About 90% of all food crops in the world are propagated by seeds, and ancient history claims that seed propagation came into being when the early man domesticated and maintained certain plants growing around him (Hartmann, 2005; Schwinn, 1994). For this reason, it is assumed that plants may have colonised land alongside their parasitic companions, giving rise to seed related diseases and reduction of productivity (Maude, 1996).

The term Seed pathology was coined by Paul Neergaard and Mary Noble in the 1940s (Nameth, 1998), but the concept as a separate discipline is believed to have originated in France in 1950 following a disease epidemic arising from the importation of disease-infested seed lot (Anselme, 1993). Although a seed related

pathogen had been discovered and established as far back as 1755 by Tillet (Maude, 1996), public interest in seed pathology as a sub-discipline of plant pathology began in the 19th century following detailed studies of seed borne pathogens carried out by the likes of Paul Neergaard (Agarwal and Sinclair, 1997).

Paul Neergaard is considered as the father of seed pathology, and he defined seed pathology as the study of diseases and deterioration of seeds caused by bacteria, fungi, nematodes, viroids and viruses, and physiological and mechanical disorders (Neergaard, 1979). Many others, after him, have defined the term seed pathology in different other ways, but all the various shades of definition promulgated could be summed up to say that Seed Pathology is the study and management of disease disorders arising from seeds, or affecting seed production and utilization (Agarwal and Sinclair, 1997; Munkvold,

2009).

Seed pathology as a sub-discipline of plant pathology, though relatively new, encompasses all we need to improve productivity, longevity, and production of disease-free breeds of seeds. The discipline is responsible for the dependence on chemicals and other techniques for improvement of seeds (Besri, 1989). In the past, seed improvement depended on application of chemicals and natural conventional means of seed maintenance like crop rotation and shifting cultivation (Besri, 1989), but the discipline has since evolved to the use of modern molecular biological techniques such as genetic engineering and polymerase chain reaction technology (Nameth, 1998).

Hence this paper reviews the history, development and emerging perspectives of seed pathology as a discipline. The findings of this work would potentially harness the successes achieved over the years on the diagnosis, treatment, prevention and control of seed related diseases and elucidate the role of the Seed Pathologists in the face of emerging new technologies.

DEFINITION OF SEED PATHOLOGY AND ITS IMPORTANCE

The entire discipline of Seed Pathology revolves around the 'biological entity' called seed. Its structure, development and function are necessary in understanding Seed Pathology in the general concept of plant pathology. Generally, the word 'seed' often refers to anything that is sown in the ground to produce a plant. However, this broad definition is rather ambiguous and grossly misleading because not everything that can be sown is actually a seed.

Like the term seed pathology, several authors have defined seed differently, essentially to mean the same thing. Some have defined a seed as the product of a ripened ovule of plants such as gymnosperms and angiosperms resulting from fertilisation and grows within the mother plant (Cain and Shelton, 2001). A seed is therefore a matured ovule; consisting the young embryo, nutrients for the embryo and is covered with a protective coat. In other words, seeds are fertilised ovules containing embryos surrounded by an integument (Maude, 1996). All these different versions of definitions put forward by different workers could be summarized to mean that a seed is a mature ovule containing an embryo, durable seed coat and endosperm. In biological terms, a seed is a small embryonic plant enclosed in a covering called the seed coat, usually with some stored food in the cotyledon.

Man has continually depended on seeds, using them in variety of ways; as food, fibres and drugs. They are also used as ornaments of beauty, feed for livestock, raw materials for industry, and planting material for cultivation of crops (Maude, 1996; Judd et al, 2002). Apart from being used as planting materials, seeds are very good

and viable sources of cooking oils, beverages, spices and other important food additives. Some are even used as beads in necklace and rosaries (Judd et al., 2002). Seeds are also used as toys by children, such as for the game conkers. As industrial raw materials, seeds of maize and other plants are used to produce industrial chemicals such as grain alcohol (ethanol) through fermentation (Judd et al., 2002).

Plant production starts with the seed and ensuring its quality therefore is an undisputable prerequisite for obtaining high yields of most plant species (Friis-Hansen, 1995). Although seed is one of the least expensive agricultural inputs, it is the most important factor influencing yield potential (Brick, 2010; Friis-Hansen, 1995) because it provides a means of survival for both itself and animal species. It is often remarked that the pace of progress of agriculture is substantially dependent on how quickly quality seeds are multiplied and marketed or distributed. This knowledge notwithstanding, rural farmers in most developing countries do have not easy access to quality seeds in their countries. Thus, it is not unusual for farmers to retain seeds after harvest so that they could be reused for cultivation in the subsequent planting season (Mumby, 2005).

As important as seeds are to human livelihood, their nature exposes them to microorganisms; some of which are pathogenic and adversely affect the physiology and metabolism of seeds, resulting to low yield and/or quality. Consequently, seed health testing, prior to sowing, is a vital prerequisite to maximize yield [International Seed Testing Association (ISTA), 1985].

Furthermore, seeds in storage are prone to insect infestation, microbial attack, unfavourable temperature conditions, etc. The incidence and severity of seed deterioration during storage problems depends on the type of storage equipment and conditions, as well as biotic factors (Vanek, 1992). Storage conditions such as high temperature and humidity encourage rapid growth of seed pests and microbial pathogens (Maude, 1996), and the activities of these organisms play very significant roles in the spoilage and loss of seeds in storage. Some fungal pathogens such as *Aspergillus*, *Penicillium*, *Fusarium* species etc whilst attacking seeds sometimes produce toxic substances or mycotoxins which may be harmful to man and animals that ingest such contaminated seeds (Judd et al., 2002).

SOME SEED-BORNE DISEASES, PATHOGENS AND THEIR EFFECTS

A variety of crops are grown in Nigeria, including cereals such as maize, wheat, rice and millets; legumes such as beans, soybeans; oil seed crops such as groundnut and other food crops are adversely affected by various seed-borne diseases (Oluwatoye, 2003).

Depending on the nature of pathogen, seeds and prevailing environmental conditions, seeds may be

infested or infected by a microorganism. Seed and seed-borne pathogens include: fungi, bacteria, viruses and nematodes (Walkey, 1991; Agarwal and Sinclair, 1997). Some microorganisms simply stick to superficial layers of seed tissues or are mixed with a seed lot. Examples of such organisms include fungi (*Ascochyta pinodella* in pea; *Drechslera sorokiniana* and *D. oryzae* in rice) and bacteria (*Pseudomonas syringae* pv. *phaseolicola* in bean, *Pseudomonas. syringae* pv. *tomato* in tomato and *C. michiganense* pv. *michiganense* in pepper) (Nome et al., 2002). These sorts of seeds with microorganisms occurring just superficially on its surface is often said to be infested or simply contaminated. In contrast to infestation or microbial contamination, some microorganisms infect seeds. In this case, the microorganism is considered to be a pathogen, and grows deep into the tissues of the seed. A seed is therefore said to be infected when a pathogen is able to penetrate deep into the tissues. Infection can be further categorized into systemic and non-systemic (Nome et al., 2002). An infection is said to be systemic if the seed-borne pathogen persists and develops with the plant as the latter progresses from the seedling stage to a fully grown plant. On the other hand, an infection is considered to be non-systemic if the pathogen remains within the seed where it causes a localized infection on the seedling at the stage of pre or post emergency (Agarwal and Sinclair, 1987). Many environmental factors directly or indirectly affect the establishment of seed-borne pathogens. Temperature, moisture, light and pH are known to be the most important environmental factors which influence the infection of plants (Tarr, 1989). Environmental factors modify the transmission of disease pathogen and their effect on host plant by modulating the action of the soil microbial community, and in the process renders a soil disease suppressive or conducive (Etebu and Osborn, 2012; Maude, 1996).

The effects of seed-borne pathogens and diseases are varied, depending on the pathogen involved, the seed itself and the prevailing environmental or storage conditions. Some of these effects are seed discolouration, seed abortion, seed rot, seed necrosis, seedling damage, crop yield and adverse effect on seed viability, germination capacity and nutritive value.

SOURCES/TRANSMISSION OF SEED-BORNE PATHOGENS

The mode of transmission of pathogens depends on the type of infection or infestation. Microorganisms that occur only superficially on the outer embryo tissues of a seed do not affect the female gametes that form the new plant and as a result, the pathogen(s) do not persist via the new plant to affect the developing seeds thereafter (Shetty, 1992). With this sort of seed-borne infection, transmission is majorly influenced by environmental factors (Shetty, 1992). In contrast, some pathogens

especially those that cause systemic infection, are transmitted directly through the tissues of the mother plant to the developing seeds. A diseased seed would usually produce a diseased seedling if the seed-borne pathogens are deep seated and the embryo area of the seed is affected (Maude, 1996). In such instances, the role of environmental factors is insignificant as the pathogen literally persists and develops with the plant as the latter progresses from the seedling stage to a fully grown plant. Sometimes infectious seed-borne pathogens are transmitted from diseased plants to infect healthy plants through indirect means. For example, a pathogen located in the inflorescence of the mother plants could be transmitted by insects, wind, rain and other means of transmission to affect the seeds and other healthy parts of the plants (Maude, 1996; Walkey, 1991). Other means of transmission are seeds contaminated with whole organisms or fungal resting bodies such as sclerotia, infected seed coats or pericarps, infected embryos, plant debris etc (Maude, 1996).

Control of seed-borne diseases

Seeds are distributed for purposes of agriculture, multiplication, conservation of germplasm or research (Kahn, 1983). Seed-borne diseases are controlled principally through one of two concepts. One is to ensure that pathogen-free seeds are planted to forestall the emergence of disease. The other is to seek ways that would eliminate disease pathogens from infected or contaminated seeds before the latter are planted.

Broadly speaking, various ways of controlling seed-borne diseases include, quarantine through appropriate legislation against disease, alleviation of disease by cultural practices, treatment of disease, and developing disease-resistant plant. The application of one or more of these principles in the prevention or elimination of infection in seeds has been shown to be effective and economically sustainable at national and international levels (Maude, 1996). Quarantine involves the separation of the diseased plant seeds, and could be achieved through one of different ways. One way would be through restriction of seeds to a given area or space. Cultural practices employed in controlling seed-borne diseases, amongst others include, crop rotation and spacing. Crop rotation improves soil structure and fertility and has been shown to reduce soil-borne diseases and pests (Anon, 1993). The growing of unrelated plant species in sequence on a particular area of land has the effect of diluting soil-borne inocula, causing pathogens and pests specific to the land to reduce with time. Shifting cultivation, mixed cropping and farming have all been shown to achieve the same purpose. Similarly, crop spacing has been shown to also affect soil-borne disease incidence and severity. Increased spacing between plants in a row or between rows of plants or between crops susceptible to the same pathogen reduces disease

spread from a seed-borne source (Agarwal et al., 1997; Maude, 1996). Sometimes varying certain planting parameters such as irrigation time, shallow planting etc. would also be cultural control measures (McGee, 1988a).

Chemical control

This involves the use of chemical fungicides directly on the pathogens like Bourdeux mixture, Thiram, Benomyl, Captan, Triazonide etc (Besri, 1989; Wang and Davis, 1997). The application of fungicides to seeds before planting serves two purposes: to control disease caused by seed-borne infection and the protection of germinating seedlings from soil borne pathogens (Besri, 1989). The number of fungicides has grown considerably, but the use of mercuric compounds to control seed-borne diseases is no longer supported because of their hazardous effects (Maude, 1996).

Seed treatment

Seed treatment is a common term which does not specify the application method but indicates that seeds are subjected, to a compound (chemical, nutrient, hormone etc.), a process (say drying) or to various energy forms e.g. heat radiation (Scott, 1990). Seed treatment objectives range from protective to preventive and then to productive in that it can protect the seed, prevent disease and produce healthy seedlings (Maude, 1996). Chemical and physical seed treatments are different applications of seed treatment (Kaufman, 2002). Whilst chemical seed treatment would include fungicidal seed treatment, physical seed treatment would include hot water treatment, dry heat seed treatment, and radiation (Kaufman, 2002).

Biological control

This is the use of micro-organisms in the control of seed-borne pathogens (Besri, 1988). Biological seed treatment involves the treatment of seeds with micro-organisms which are beneficial to plant growth and/or achieve disease control, (McGee, 1988a). Seed-treatment machinery includes Auger-mixing, drum-mixing, spinning disc distribution, film-coating, pelleting of seeds, etc. (Maude, 1996).

Breeding for resistance

Some varieties of seeds are resistant to diseases but lack other desirable commercial and edible qualities, while those with commercial and edible qualities are often susceptible to diseases. When a resistant variety is bred with the non-resistant, healthy varieties with good edible qualities are produced. Breeding for resistance has been

proven effective in controlling *Verticillium* wilt in various crops and might be expected to eliminate or reduce the pathogen on seed (Besri, 1988).

SEED HEALTH TESTING

Health of seed refers primarily to the presence or absence of disease-causing organisms or deficiencies. The procedures for seed health testing include: quarantine, evaluation of planting value, certification scheme, availability of seed treatment, storage quality, resistance of cultivars, etc (Anon, 1993). As far back as 1993, the most common and reliable seed health testing technique are the incubation tests (Anon, 1993).

Current techniques for detection of seed pathogens include direct inspection of seeds both macroscopically and microscopically, and involve the following methods:

Blotter method

This is one of the incubation methods where the seeds are planted on a well water-soaked filter paper, and incubated for 7 days at 20°C followed by microscopy (Anon, 1993).

Agar plate method

Here, surface disinfected seeds are plated on an agar medium and the plated seeds are usually incubated for 5 to 7 days at 22 to 50°C. After which identification is done based on colony characteristic and morphology of sporulating structures under a compound microscope (Anon, 1993). Other specific methods are adopted, depending on the type of microorganism, and include the following:

1. Staining methods are used for seed-borne biotrophs which cannot be grown out on a nutrient (agar) or basal (blotter) substrate (Anon, 1993)
2. Extraction: attempts to optimise the recovery of the target organism.
3. Isolation: Microorganisms are isolated by plating small volumes of the extraction medium onto general or semi-selective agar media (Schaad, 1988).

These traditional method are time-consuming and do not allow for accurate identification of the pathogen to the species level. Hence nucleic acid based molecular techniques are being clamoured for in seed health testing, and are being developed (Dreaden et al., 2012).

Serology

This method is based on the immunological principles

Table 1. General features of seed detection assays including the time required for completion, sensitivity, ease of application, specificity, and applicability for the detection of seed pathogens.

Assay specificity	Time required	Sensitivity	Ease of application	Specificity
Visual examination	5-10 min	Low	Simple and inexpensive (requires experience)	Low
Semiselective media	2-14 days	Moderate	Simple and inexpensive	Low-moderate
Seedling grow-out assay	2-3 weeks	Low	Simple, inexpensive and robust	Low
Serology	2-4 h	Moderate-high	Simple, moderately expensive and robust	Moderate-high
Conventional DNA extraction and PCR	5-6 h	High	Complicated; easy to interpret, expensive	Very high

Source: Walcott (2003).

where foreign molecules (that is, immunising agents or antigens) injected into the blood stream of mammals stimulate the immune system of those mammals to produce specific antibodies which will recognise and bind to the antigens (Schaad, 1988).

Such antibodies recognise many chemical sites, referred to as epitopes on target antigens. ELISA (Enzyme Linked Immunosorbent Assay) modified for the detection of seed-borne viruses has had considerable impact on the diagnosis of virus and bacterial diseases (Lagerberg, 1996). The ELISA technique is based on the use of polystyrene microtitre plates and is as follows:

A specific antibody is absorbed to the well to which a test sample including the pathogen is added, followed by enzyme-labelled specific antibody and the enzyme substrate leading to a coloured product in proportion to concentration of the pathogen (Fox, 1993).

The ELISA method was in vogue for quite a long while in the past, especially with reference to the inspection of plant quarantine viruses (Stein, 1979). Unfortunately the method is laden with issues of false positive reaction and low sensitivity (McGee, 1995; Caruso et al., 2003; Priou et al., 2006). As a result, molecular biological methods which are simpler and highly sensitive are at present the preferred choice in seed health testing procedures (Fonseca et al., 2005; Lee et al., 2013; Majumder et al., 2013; Munkvold, 2009; Park and Kim, 2004; Peiró et al., 2011).

Molecular biological method

Another innovative method in the detection of seed-borne pathogens is the use of molecular biological techniques such as the Polymerase Chain Reaction (PCR). This nucleic acid-based assay allows Researchers to detect very minute amounts of specific DNA sequence on the surface of or internal to a seed (Nameth, 1998). PCR is a biochemical method that amplifies the concentration of the target sequence of DNA by up to a million fold within a very short time, thereby transforming previously undetectable amounts into detectable quantities (Barjet,

2011; Etebu, 2013). Amplification involves the two primers which flank the DNA segment to be amplified and exposed to repeated cycles of heat denaturation of the DNA, annealing of the primers to their complementary sequences and extension of the joint primers with the DNA polymerase enzyme (Etebu, 2013).

Owing to the high sensitivity and specificity of molecular based seed testing approaches over other known methods (Table 1), numerous PCR-based assays have been reported for seedborne pathogens in the last over 2 decades (Audy et al., 1996; Dreaden et al., 2012; Frederick et al., 2002; Glynn and Edwards, 2009; Wen and Zhang, 2012; Zhang et al., 1999).

Notwithstanding the potential improvements over conventional assays, PCR-based seed assays were not widely adopted for two basic reasons, until quite recently. Firstly, many seed types contain compounds such as tannins and phenolic compounds that inhibit DNA amplification when PCR is attempted directly on seed extracts, and secondly, PCR was expensive (Walcott, 2003). However, these fears and potential limitation of PCR have been substantially addressed and remedies adopted (Etebu, 2013). In particular to PCR seed testing techniques, modified forms of PCR such as enrichment or BIO-PCR, immunomagnetic separation (IMS) and magnetic capture-hybridization (MCH) have been developed to overcome the limitations encountered in detecting seedborne pathogens through the use of molecular based techniques (Walcott, 2003).

SEED CERTIFICATION

Seed certification is carried out to ensure seed quality. In Nigeria, seed certification is done by the National Agricultural Seed Council (NASC) under the auspices of the Federal Ministry of Agriculture. One of the functions of NASC is to analyze and propose programs, policies, and actions regarding seed development and the seed industry in general, including legislation and research on issues relating to seed testing, registration, release, production, marketing, distribution, certification, quality

control, supply and use of seeds in Nigeria, importation and exportation of seeds, and quarantine regulations (FRN, 1992). However, this function among others, have suffered major setbacks due to non-implementation of legislations (Khare, 1988). Normally, seed undergo certification procedures after which they would be deemed fit for the intended use. Research institutes responsible for seed certification should be active and carry out field inspection of seed sources, inspection and testing of seed lots, and a whole lot of function which are not obtainable in Nigeria as farmers' retained seeds are strictly used. Seed that has passed the field inspection and laboratory analysis can be tagged as certified (Elias et al., 2011).

ROLE OF A SEED PATHOLOGIST

The roles of a seed pathologist varies among stations due to traditions, facilities, testing needs, educational standard of the pathologist, and plant pathological activities at other institutes (Jorgensen, 1988). The duties of a seed pathologist will usually be to organise seed disease testing, other kinds of testing such as testing for quality of seed treatment, testing fungicides for seed treatment and testing for resistance (Jorgensen, 1988).

CONCLUSION

The vigour and health of seeds is a strong determinant of the quality, uniformity and yield of the growing crop. To achieve the full potential of seeds and to minimise loss due to diseases, Seed Pathology is a promising and important discipline in the quest for food security. The primary aim of Seed Pathology is to control seed-related diseases, develop new technologies that would improve quality of seeds, reduce seed loss and enhance food production. As a meeting point between Microbiology and Botany, Seed pathology uses the knowledge of both disciplines to control pathogenic micro-organisms and improve seed production.

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