Detection of seed borne pathogens in wheat: recent trends

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Abstract

Infected wheat seeds are the carrier of pathogens for long-distance dissemination. Major impact of seed borne diseases in wheat is not only the yield reduction but also deteriorate marketable quality of grains. Early detection of pathogens is a crucial step in diagnosis and management programmes in wheat. The failure to adequately identify and detect plant pathogens using conventional, culture based morphological techniques has led to the development of nucleic acid-based molecular approaches. Polymerase chain reaction (PCR) revolutionised pathogen detection and identification, but these methods have not yet entirely replaced traditional cultural and phenotypic tests practiced for detection of major wheat seed borne pathogens e.g. NaOH seed soak method for detection of *Tilletia indica* causing Karnal bunt of wheat. Methods for detection of seed borne microorganisms in wheat are ranged from simple visual observation to spectroscopic and imaging techniques. Immuno-diagnostic tools can also be successfully employed for differential diagnosis, disease surveillance of seed borne pathogens of quarantine importance and determination of teliospore load in wheat seeds. PCR-based methods result in high level of sensitivity, specificity is used for species-specific detection of *Tilletia* spp *and Fusarium* spp. The present paper highlights the brief outlines of traditional detection methods and discusses recent PCR-based and spectroscopic and imaging techniques being employed in detection of seed borne pathogens in wheat

Keywords: Detection; karnal bunt; loose smut wheat diseases; PCR-based assay; seed borne.
Abbreviations: EPPO-European and Mediterranean Plant Protection Organization; ISTA- International Seed Testing Association; IFST- immunofluorescence staining test; SIBA- seed immunoblot binding assay; RAPD-Random Amplified Polymorphic DNA; NIR-Near Infrared; SAM-Spectral Angle Mapper; PCA-Principal component analysis; PCR-Polymerase chain reaction; ELISA-Enzyme linked immunosorbant assay; ITS-Internal transcribed spacer.

Introduction

Wheat (*Triticum aestivum* L.) is served as a source of staple food to the mankind since times immemorial. Seed borne diseases of wheat like Karnal bunt (*Tilletia inidica)*, loose smut (*Ustilago nuda* tritici), head blight or scab (*Fusarium* spp*)* and tundu or ear cockle (*Clavibacter tritici* and *Anguina tritici*) are considered as the constraints in wheat cultivation, that affect crop yield and grain quality (Mitra, 1931; Agarwal et al., 2008; Kumar et al., 2008). In addition, diseased seed may have toxic effects on humans and animals. The major impact of Karnal bunt of wheat is on quality and not yield reduction (Brennan et al., 1990). Infected seeds are the most important carrier of pathogens for trans-regional and long-distance dissemination. Karnal bunt was introduced into United States (US) and other bunt-free countries because of several interceptions of teliospores in wheat shipments entering the US. from Mexico (Matsumoto et al., 1984), has become a significant concern to the US wheat industry (Smith et al., 1996). The movement of wheat into US. and other Karnal bunt free countries is the subject of strict quarantine regulations (Royer and Rytter, 1988). Among all seed borne pathogens, fungal pathogens hold more economic importance than bacterial and nematode pathogens (Joshi et al., 1986; Kulkarni and Naragund, 1986; Mathur and Cunfer, 1993; Sarri, 1986; Sharma et al., 1998). Detection and management of seed borne diseases through quality control programmes that monitor seeds from harvest to purchase, marketing and sowing in the field are essential to ensure high quality, pathogen free and genetically pure seed. Detection of theses pathogens is based primarily on conventional methods

*viz.,*direct inspection of dry seeds, washing test, soaking test, incubation tests, blotter tests, embryo count test and filter and centrifuge extraction technique (Castro et al., 1994). These methods had been in regular practice till last decades of 20th century. Nucleic acid based molecular approaches (Chahal and Pannu, 1997; Lopez et al., 2003), which are specific, rapid and reliable, were developed for accurate and rapid detection of seed borne pathogens. Molecular diagnostics began to develop a real momentum after the introduction of PCR in the mid 1980s. Diagnostic assays based on PCR have been developed for major seed borne diseases of wheat. Rapid development of genomic techniques for characterization of plant pathogens of the past decades has greatly simplified and improved pathogen detection and identification. PCR-based assays are practiced for species-specific detection of *Fusarium culmorum, F. graminearum* and *F. avanaceum* from contaminated seeds (Schilling et al., 1996). Detection through real-time PCR was reported for *Fusarium* spp (Heid et al., 1996; Waalwijk et al., 2004). This paper deals with recent trends in detection of pathogens from wheat grains. A brief outline of conventional and other methods are also being highlighted.

Major seed borne diseases of wheat

Karnal bunt (Tilletia indica)

Karnal bunt affects mainly common wheat, durum wheat, triticale and other related species (Royer et al., 1986;

Warham et al., 1986). The disease reduces seed quality, inflicts changes in the chemical composition of infected grains, and renders seed useless for consumption. Severely infected grains show considerable reduction in the viability of seeds, (Rai and Singh, 1978). There is a progressive decline in fertility with increasing grades of infection (Mitra, 1935; Munjal and Chatrath, 1976). Symptoms of the disease become evident only when the grains develop fully. The disease is first noticed when broken smutted kernels are seen in threshed grain. This non systemic pathogen is spread by airborne sporidia at the time of flowering, resulting infection leads to partial bunting of wheat kernels and a characteristic fishlike odour which emanates from infected grains (Warham et al., 1986). Fungal hyphae concentrate in the inflorescence and spikelets, transforming the ovary into smutted tissues. The disease has been reported to occur in other parts of the world such as Pakistan, Nepal, Iraq, Iran, Afghanistan and Mexico (Begum and Mathur, 1989; Gill et al., 1983; Joshi et al., 1983; Matsumoto and Bell, 1989; Singh, 1994; Warham, 1986). It was reported to occur in several limited areas of the Southwestern US (Bonde et al., 1997; Ykema et al., 1996) and now Karnal bunt pathogen is considered as a major threat to the wheat industry of the USA (Anon, 1991). The movement of wheat seeds from one country to another is strictly regulated through quarantine measures to restrict the entry of Karnal bunt infected wheat grains. Therefore, Karnal bunt acts as a genuine non-tariff trade barrier in commercial seed trade in the international markets and thus threatens wheat trade because export of Karnal bunt infected wheat grains would disseminate seed borne pathogens within and between regions, countries and continents (Kumar et al., 2008). In China, to get rid of introduction of Karnal bunt from other countries only wheat lots declared as having zero infection levels are imported. In the USA, there is concern about the possibility of export losses due to the introduction of Karnal bunt in Arizona, Texas and New Mexico (Ykema et al., 1996). The USA has declared *T. indica* as a quarantine pest. The European and Mediterranean Plant Protection Organization (EPPO/EOEPP) has imposed several standards for regulating diseases in the international trade. Regulatory measures have been suggested for isolation, sampling as well as for diagnostics schemes including morphological comparisons and molecular confirmations with reference to *T. indica* which was listed under the category of plant pathogens can be used as biological weapons (Beck, 2003). Seed scientists have developed techniques for analysing large number of samples for seed certification and plant quarantine. The advent of newer molecular diagnostics based on nucleic acid and immunological reaction permit precision detection of Karnal bunt (Kumar et al., 1998).

Head (ear) blight or scab (Fusarium spp)

A complex of *Fusarium* spp. causes head blight or scab on wheat, results not only in yield loss, but also causes depreciation of the harvested product due to the accumulation of toxins such as deoxynivalenol produced by *Fusarium* spp. (Nedelnik et al., 2007). Time of flowering is very much susceptible period for primary infection. Initial infection is characterized by whitening and drying of individual spikelets or entire parts of the ear. Florets become infected just after blooming, grains are often not formed, while other infected spikelets have grains with various deformities, shrunken and with pink-white coloration (Klem and Tvaruzek, 2005). *F. poae* was identified as one of the major pathogen causing head blight in wheat. Parry et al. (1995) highlighted that up to 17 *Fusarium* spp. have been associated with ear blight, but

F. graminearum (*Gibberella zeae*), *F. avenaceum* and *F. poae* were most common.

Loose smut (Ustilago nuda var. tritici)

Loose smut manifests itself only when the ears emerge from the boot leaf, usually a few days earlier than healthy ones. Initially, diseased heads are black and clearly visible among newly-emerged, green, healthy heads. Later, the whole head is transformed into a dry mass of spores, blown off by wind (Rangaswamy and Mahadevan, 2008). The teliospores germinate on the flowers. Mycelium invades part of the seed embryo, thus the fungus survives as mycelium inside the seed, so internally seed borne in nature.

Tundu or ear cockle or yellow ear rot disease (Clavibacter tritici and Anguina tritici)

The disease has been reported from Egypt, parts of Australia and China. Disease is caused by the combined action of the bacterium *Clavibacter tritici* and the nematode *Anguina tritici* and is characterized by production of yellow slime on the stem and inflorescence, which dries up to form sticky yellow layers and cause curling and twisting of the spikes. Most of the grains in the earhead are replaced by galls formed by the nematodes, carry also the causal bacterium. Disease causing nematode can remain dormant in the grains having galls over a period of more than 10 years and is capable to cause infection.

Other seed borne diseases

Septoria blotch (*Septoria tritici*, *S. nodorum* and *S. avenae* f. sp. *triticea*), spot blotch (*Helminthosporium sativum*), tan spot (*H. tritici-repentis*), Alternaria leaf blight (*Alternaria triticina*), snow mold (*F. nivale*), (*Stagonospora nodorum*) leaf and glume blotch (*Stagonospora nodorum*), ergot (*Claviceps purpurea*) and black chaff or bacterial streak (*Xanthomonas campestris* pv. *translucens*) are the other seed borne diseases in wheat.

Detection methods of seed borne pathogens

The selection of a method depends upon the purpose of the test *i.e.* whether the seeds are to be tested for seed certification, seed treatment, quarantine etc. If for quarantine purposes, then highly sensitive methods are preferred, which can detect even traces of inoculum. Methods that have been employed for detection of seed borne diseases of wheat are conventional methods, nucleic acid based techniques and spectroscopic and imaging techniques.

Conventional methods

Some classical diagnostic methods have been standardised in last century and have been applied to seed samples for a long time. They are as follows.

Visual inspection of seeds

Fungal structures (sclerotia, encrusted mycelium) and/or particular effects of pathogens on the seed surface (discoloration, pigmentation, etc.) are detected by naked eye or by the use of optical lenses (De Tempe and Binnerts, 1979). Yellow ear rot or tundu disease infected wheat seeds

can be detected by visual inspection of black galls which are due to nematode *Anguina tritici (Steinbuch) Chitwood.*

Discolouration of seeds

Appearance of galls or cockles caused by nematode *A. tritici* (Steinbuch) Chitwood on wheat seeds are small, dark purplish black or dull. The diagnosis is confirmed by soaking galls in water for 1 hr and cutting them into pieces in drops of water. Active larvae are released into the water and can be seen under a binocular microscope.

Morphological Abnormalities

Infected seeds of wheat can easily be detected by examining seed samples in day light. e g. formation of bunt balls by *T. tritici* (common bunt) on seeds. The range of bunt infection varies from the small area adjoining the embryo tip to complete conversion of the seed into a black powdery mass of teliospores (Mathur and Cunfer, 1993). Sometimes masses of teliospores exude and remain adhering to the rice glumes.

Microscopic examination

Infected seeds under a stereomicroscope reveal the presence of spores on seed surfaces. Wheat seeds infected with Karnal bunt can be detected by observing the seeds very carefully under a stereomicroscope at 12.25 X magnification (Begum and Mathur, 1989). Further, the contaminated seeds may be subjected to the washing test to confirm the identity of the pathogen.

Washing test

The washing test is useful for rapid detection of surface borne teliospores of *T. indica* and *T. barclayana* (Shetty et al., 1988; Begum and Mathur, 1989). Spores are washed from seeds in water and the suspension is then centrifuged, the supernatant is discarded and the pellet is resuspended (Mathur and Cunfer, 1993). This suspension is then examined under a compound microscope for the presence of teliospores (Begum and Mathur, 1989).

Seed soak method

Bunted seeds can be detected by using the NaOH soak method (Agarwal and Verma, 1983; Agarwal and Srivastava, 1985). The seeds are soaked in 2% NaOH solution for 20 hr at 20°C. The infected portion of the seeds appears jet black and shiny, in contrast to the pale yellow healthy seed. When black seeds are ruptured in a drop of water, a stream of teliospores is released. The NaOH treatment increases the colour contrast between diseased and healthy seeds (Agarwal and Mathur, 1992). Seeds infected by tundu disease can also be detected by overnight soaking of galls in water. Thousands of juvenile nematodes (*A. tritici*) can burst from the gall upon piercing with a needle.

Filter and centrifuge extraction technique

In this method, described by Castro et al. (1994), a sample of seeds is washed in water containing 0.001% Tween 20. The wash water is filtered through a 60 μm diameter pore size screen and then through 12 μm pore size membrane. The first wash separates out debris and the second teliospores. The spores are then washed from the membrane, pelleted by centrifugation and examined under the light microscope for

identification of the spores. Even five spores per 100 seeds can be detected.

Incubation method

Incubation methods are the most popular and frequently used for the detection of a great number of seed-transmitted pathogens (De Tempe and Binnerts, 1979). Two methods *viz.,* blotter and agar plate are recommended by ISTA for routine examination of crop seeds infection. The detection of *A. triticina* in seeds may be facilitated by plating disinfested seeds on the medium recommended by Agarwal et al. (1993). Teliospores viability can be tested by assessing germination on 2% water agar (Chahal and Mathur, 1992). Single teliospore cultures can be characterized (Micales et al., 1986; Bonde et al., 1989). Large differences in the isozymes of *T. indica* and *T. barclayana* distinguish these two species.

Embryo count method

Rennie (1982) employed this method for detecting loose smut pathogen *Ustilago nuda* var. *tritici* Schaf. When the inoculum of the fungus is located in the embryo, the separation of the embryo from the rest of the seed for microscopic observations is necessary. The method has been improved avoiding the use of phenol and reducing the number of embryos examined per seed sample (Khanzada et al., 1989; Cappelli and Covarelli, 2005).
The conventional methods are mostly based on interpretation of visual symptoms, culturing and laboratory identification. They require extensive taxonomic expertise and very much time consuming. They are not much reliable and difficult for samples with hidden symptom.

Immunodiagnostic methods

Since immunodiagnostic methods are most efficient they are successfully employed exclusively for the detection and diagnosis of Karnal bunt. Kumar et al. (2008) developed antibody methods such as microtitre enzyme linked immunosorbant assay (ELISA), immunofluorescence staining test (IFST), seed immunoblot binding assay (SIBA), dyed latex bead agglutination test and immunodipstick assay. These immunoassay systems could be allowed for reliable quantitative assessments in a high throughput manner in regulatory laboratory facilities if developed in an appropriate rapid format in the form of kits such as immune-dipstick and immune-lateral flow assays. The immunodiagnostic assays for field use are inexpensive, rapid and do not require highly trained personnel.

Micro-titre ELISA is being used for early detection of Karnal bunt pathogen in the host when the infection levels are very low (Varshney, 1999). SIBA was developed by Kumar et al. (1998) to check out the teliospore load on seeds at the time of vigour testing. The principle behind this technique is that when the infected seeds are kept on the nitrocellulose membrane, the teliosporic antigens are adsorbed and diffused according to the teliosporic load on the nitrocellulose sheet. The coloured imprint develops and appears to have direct correlation with the grade or severity of infection on bunted grains.
Gupta et al. (2001) developed *IFST* in order to detect the intact teliospores found in sori of the infected seeds or as fungal contaminants on the infected seeds or in the soil. The procedure is based on the principle of indirect ELISA. The

teliospores can be seen as bright green, patchy and ring-shaped fluorescence under the microscope.

Immunodipstick test is much sensitive and can detect even a very small number of teliospores (Kesari and Kumar, 2002). It includes the use of nitrocellulose padded plastic tags. When teliospores are kept on nitrocellulose pads, the torer-off teliosporic antigens are adsorbed due to the charged nature of the membrane.

Kumar et al. (2008) developed the dye latex bead agglutination test which is considered better for detection of solubilised teliosporic antigens over intact teliospores. The teliosporic antigens are solubilised using sonication and detergent extraction and are allowed to adsorb on the surface of blue colour dyed latex beads, which are then immunoprocessed using polyclonal anti-teliospore serum.

Among all these immunodiagnostic methods, easier ways to detect Karnal bunt at field level are through an immunodipstick assay and a dyed latex bead agglutination test if appropriate rapid formats are developed and commercialized (Kumar et al., 2008).

Nucleic acid based techniques

Among the tools available for pathogen detection, nucleic acid based techniques are widely recognized as one of the most useful and efficient methods for detection. Nucleic acid based detection techniques, particularly those that rely on the PCR, typically are rapid, specific and highly sensitive. However, proper implementation of these techniques poses challenges, ranging from the significant cost of the technology to the need to understand the techniques well enough to be able to interpret.

PCR based methods

PCR is an *in vitro* method of nucleic acid synthesis by which a particular segment of DNA can be amplified which detect organism specific DNA / RNA sequence. It involves two oligonucleotide primers that flank the DNA fragment to be amplified and repeated cycles of heat denaturation of DNA, annealing of the primers to their complementary sequences and extension of the annealed primers with a thermostable DNA polymerase (Mullis, 1987). PCR is used to create large amount of copies of DNA. Several attempts have been made to develop species-specific PCR primers for fungal plant pathogens (Hensen and French, 1993). Fungal mitochondrial DNA has been widely used as a source of molecular markers for evolution (Bruns et al., 1991), taxonomy (Martin and Kistler, 1990) and genetic diversity studies (Forster and Coffey, 1993).

Detection of T. indica

Smith et al. (1996) used PCR to identify *T. indica*. The method used two sets of oligonucleotide primers developed by sequence analysis of cloned *Dra*I fragments of mitochondrial DNA of *T. indica*. The primer pair TI17M1 (5'-TCCCCTTGGATCAGAACGTA-3') and TI17M2 (5'-AGAAGTCTAACTCCCCCCTCT-3'), derived from clone Pti-md17, amplified a single 825-bp product from all isolates of *T. indica* and no products for other *Tilletia* spp. In addition, the primer pair T157M1 (5'-TTTTCCCTCTCTCCTTTTTTCA-3') and TI57M2 (5'-AGCAAAGACAAAGTAGGCTTCC-3'), derived from clone pTI-MD57, produced a product of 118 bp which was unique to *T. indica*. Specificity of the primers was evaluated using 78 isolates of *T. indica* and 79 isolates of five other

Tilletia spp., including 69 isolates of *T. barclayana*, from geographically diverse locations. The specificity of amplification products for *T. indica* was confirmed by Southern-blot hybridization using pTI-MD17 or pTI-MD57 as ³²P-labelled probes. Ferreira et al. (1996) and Smith et al. (1996) designed PCR primers from mitochondrial DNA of *T. indica* isolates. These primers were specific for *T. indica* and not other *Tilletia* species. This approach was used to identify germinating teliospores and much useful to identify only *T. indica* from *T. barclayana* mixed wheat seeds. Frederick et al. (2000) developed PCR assay to differentiate *T. indica* from *T. walkeri*, bunt pathogen of ryegrass. Five sets of PCR primers were made specific to *T. indica* and three sets were designed specifically for *T. walkeri* based upon nucleotide differences within the mitochondrial DNA region. In addition, a 212 bp amplicon was developed as a target sequence in a fluorogenic 5' nuclease PCR assay using the TaqMan system for the detection and discrimination of *T. indica* and *T. walkeri*.

Detection of Fusarium spp

F. poae

Parry and Nicholson (1996) carried out random amplified polymorphic DNA (RAPD) assays on a range of isolates of *F. poae,* head blight pathogen to identify markers common to all isolates. Two fragments were isolated, cloned and used to probe Southern blots of DNA from *F. poae* and isolates from a range of wheat seed and stem base pathogens. One fragment, which hybridized preferentially to DNA of *F. poae* was partially sequenced and two pairs of primers (Fp8 F/R and Fp82 F/R) were generated to use in the PCR. Amplification of target DNA occurred following PCR of all isolates of *F. Poae*, but not from any of a range of other fungal species associated with diseases of cereal ears and seed. The primer pair Fp82 F/R was used to detect *F. poae* in seed extracts. This system offers the potential to determine the presence of *F. poae* in wheat and avoid problems commonly associated with conventional diagnosis of the disease.

F. avenaceum by RAPD PCR

Existing PCR-based assays for the detection of *F. avenaceum* were found to cross-react with *F. tricinctum*. When RFLP differences in the internal transcribed spacer (ITS) regions surrounding the nuclear 5.8S rDNA were insufficient to discriminate between isolates of the two species, they were clearly differentiated through RAPD profiling by Turner et al. (1998). RAPD fragments from *F. avenaceum* isolates were screened for hybridization to *F. tricinctum* DNA on Southern blots. One of 12 selected RAPD fragments showed no hybridization to genomic DNA from *F. tricinctum*. This fragment was cloned and sequenced and the sequence obtained was used to design PCR primers. The primers were found to be specific for *F. avenaceum*, with no cross-reactions obtained with *F. tricinctum* or any other wheat pathogen assayed. The primers were able to differentiate between the two species in infected plant material, in contrast to the earlier days.

Species specific detection of F. culmorum, F. graminearum and F. avenaceum by RAPD PCR

Schilling et al. (1996) developed differential detection assays employing PCR amplification of sequence-characterized amplified regions for *F. culmorum, F. graminearum* and *F.*

avenaceum. Unique fragments from RAPD profiles that differentiated *F. culmorum* and *F. graminearum* were cloned and sequenced. Based on the sequences, pairs of 20-mer oligonucleotide primers were designed to yield distinguishable amplicons of different molecular weight. Single fragments were amplified with each of the primer pairs that were specific to *F. culmorum* and *F. graminearum.* Screening a broad range of isolates of *Fusarium* spp., other cereal pathogens and potential host plants revealed no significant cross-reactions for any assay. The assays were capable of detecting less than 10^{-12} g of fungal DNA and enabled the detection of individual *Fusarium* spp. directly in extracts of infected stem tissues and grains of wheat showing disease symptoms. Additionally, ITS of nuclear ribosomal DNA were amplified with universal primers and analyzed for sequence variation among the species. The ITS sequences of *F. culmorum* and *F. graminearum* were not polymorphic enough to design species-specific primers. ITS-1 and -2 of both species were compared to those of *F. avenaceum* and revealed sufficient sequence variation, especially in ITS-2, to derive primers for specific amplification of *F. avenaceum.* These specific, sensitive PCR assays represent valuable, versatile new tools for diagnosis, epidemiology, screening of breeding materials for *Fusarium* resistance and fungal population genetics.

Fusarium spp

Nedelnik et al. (2007) determined frequency of occurrence of four *Fusarium* spp. on wheat *viz., F. graminearum, F. culmorum, F. avenaceum* and *F. poae* in the Moravia region, Czech Republic by using PCR methods. They detected the species by using one of the methods of PCR; for *F. avenaceum* the method of Turner et al. (1998) was used, *F. culmorum* and *F. graminearum* were detected according to Schilling et al. (1996), while *F. poae* was detected using the method of Parry and Nicholson (1996).

Multiplex PCR

Multiplex PCR allows the simultaneous and sensitive detection of different DNA or RNA targets in a single reaction. It helps in reducing the number of tests required (James et al. 2006), but care is needed to optimize the conditions so that all amplicons can be generated efficiently. Fraaije et al. (2001) developed a multiplex PCR assay to detect and quantify four foliar fungal pathogens in wheat. For *S. tritici* (leaf blotch) and *S. nodorum* (leaf and glume blotch), the β-tubulin gene was used as the target region.

Real time PCR

Recently, a TaqMan real-time PCR (Heid et al., 1996) method was developed to quantify different species of *Fusarium* in wheat kernels (Waalwijk et al. 2004).

Spectroscopic and imaging techniques

Recent developments in agricultural technology have lead to a demand for a new era of automated non-destructive methods of plant disease detection. The spectroscopic and imaging techniques are unique disease monitoring methods that have been used to detect diseases and stress due to various factors, in plants and trees. Current research activities are towards the development of such technologies to create a practical tool for a large-scale real-time disease monitoring under field conditions. The spectroscopic and imaging techniques could be integrated with an autonomous agricultural vehicle that can provide information on disease detection at early stages to control the spread of plant diseases.

Reflectance or transmittance spectroscopy

Reflectance or transmittance spectroscopy, either in the visible or the near infrared (NIR) range has been used to measure several single kernel characteristics. Dowell (1998) used NIR reflectance to classify red and white wheats. Measuring protein content of single wheat kernel is described by Delwiche and Hruschka (2000). Dowell et al. (1998) used NIR reflectance to measure internal insect infestation of wheat kernels. Dowell et al. (1999) predicted scab, vomitoxin and ergosterol in single wheat kernels using NIR spectroscopy. Pettersson and Åberg (2003) described the application of NIR spectroscopy for measuring mycotoxins in cereals.

Image analysis

Image analysis is another method for fast non-destructive characterization of single kernel. Several machine vision applications use color and geometric features for cultivar identification (Keefe and Draper, 1986; Chtioui et al., 1996; Zayas et al., 1986; Neuman et al., 1989). Ng et al. (1998) described measurements of mechanical or mold damage using image analysis. Nielsen et al. (2003) investigated a combination of image analysis, NIR transmission spectroscopy and several other techniques to predict kernel protein, vitreousness, density and hardness. Ruan et al. (1998) used machine vision in combination with a neural network to estimate predicted scab.

Hyper-spectral imaging for detection of head blight

Recently, spectral imaging sensors have become available. These sensors combine image analysis with spectroscopic techniques. Delwiche and Kim (2000) described the application of hyperspectral imaging to detect *Fusarium* head blight in wheat. The sensor in this application possesses a wavelength range of 425 to 860 nm. Based on reflection measurements of normal and head blight damaged kernels of three different varieties, wavelength bands were selected to separate normal and damaged kernels. Recently, Bauriegel et al. (2011) used hyper-spectral imaging analysis for early detection of head blight pathogen *Fusarium.* Occurrence of head blight can be detected by spectral analysis (400-1000 nm) before harvest. With this information, farmers could recognize *Fusarium* contaminations. Wheat plants were analyzed using a hyper-spectral imaging system under laboratory conditions. Principal component analysis (PCA) was applied to differentiate spectra of diseased and healthy ear tissues in the wavelength ranges of 500-533 nm, 560-675 nm, 682-733 nm and 927-931 nm, respectively. Head blight could be successfully recognized during the development stages. With the imaging analysis method, 'Spectral Angle Mapper' (SAM) the degree of disease was correctly classified (87%) considering an error of visual rating of 10%. However, SAM is time-consuming. It involves both the analysis of all spectral bands and the setup of reference spectra for classification. The application of specific spectral sub-ranges is a very promising alternative. The derived head blight index, which uses spectral differences in the ranges of 665-675 nm and 550-560 nm, can be a suitable outdoor classification method for the recognition of head blight.

Other methods

Several chemical methods exist to detect *Fusarium* head blight damaged kernels. Chemical tests such as liquid chromatography (Rice et al., 1995; Sydenham et al., 1996); gas chromatography-mass spectroscopy (Pestka et al., 1994) thin-layer chromatography (Schaafsma et al., 1998) and high pressure liquid chromatography (Pestka et al., 1994) are developed to detect damaged kernels or mycotoxin deoxynivalenol levels produced by *Fusarium* spp. in wheat.

Conclusion

Currently, more and more diagnostic laboratories and inspection agencies are using molecular methods for detection and identification of seed borne pathogens of wheat. The development of more versatile robust and cost effective systems, allowing for greater sensitivity and specificity, elevated throughput and detection of multiple microbes will continue over the coming years. Universal applicability of a new nucleic acid based assay may lead to abandon traditional reliable methods. For some laboratories, the need for chemicals, equipments and lack of skill workers are drawbacks in adopting PCR-based detection methods for routine detection. Both domestic and international plant quarantine stations must be facilitated with high throughput, rapid and sensitive methods for detection of quarantine pathogens. Novel approaches including biosensors or an electronic nose to detect quarantined pathogens in an entire seed lot, are prospects for the future (Alvarez, 2004). Perhaps advances will be made in nanotechnology (Basu et al., 2004) such that microscopic nanoprobes will crawl across individual seeds to detect and destroy pathogens.

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