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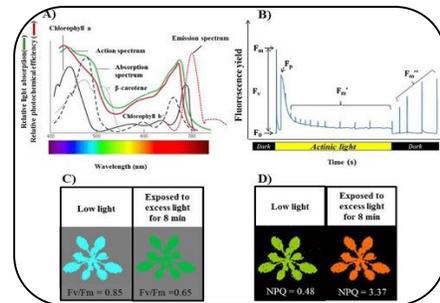
CHLOROPHYLL FLUORESCENCE SPECTRAL IS PERFECT TOOL FOR DETECTION OF PLANT DISEASES

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ABSTRACT:

Market pressures have fueled the demand to address the growing incidence of *Fusarium* head blight (FHB) in cereal production, particularly in wheat. The symptoms of this disease can be clearly identified by image analysis. This technique can therefore be used to map the incidence and extent of *Fusarium* infection. From this point of view, a separate harvest can be considered in the field. Characteristics, requirements, and limitations of detecting *Fusarium* on wheat, in the field and in the laboratory, based on the use of chlorophyll fluorescence imaging, are discussed.



KEYWORDS : *Fusarium* head blight (FHB) , Characteristics, requirements , image analysis.

INTRODUCTION:

The goal of modern agriculture is not only to increase and optimize production, but also to produce safe and healthy food and high-quality feed. In this context *Fusarium* infection represents an important, increasing problem, especially on cereals. This problem has greatly increased in recent years due to promoting cultivation systems such as *Fusarium* maize to increase yield. As a result, the prevalence of *Fusarium* infection has increased worldwide. Income loss of up to 30% can represent the overwhelming impact of the disease. Typical early and outward symptoms of *Fusarium* infection are bleaching of individual spikelet's and partial death of ears or heads before maturity. These symptoms are synonymous with the name of this fungal disease: "head blight". A direct consequence of *Fusarium* infection is the eventual development of shrunken Loma's tombstone kernels leading to massive crop failure. The worst problem with the disease, however, is the potential toxic side effects due to the production of mycotoxins. Highly contaminated grain is harmful and dangerous to humans and livestock. *Fusarium* produces varying amounts of mycotoxins such as deoxinivalenol (DON), zearalenone and fumonisins, which can cause vomiting, mass loss, kidney failure, abortion, false pregnancy and cancer.

Therefore, infected grains should always be excluded from the human food cycle or livestock feed. In this context, the detection of *Fusarium* wilt in the field by simple and rapid methods will lead to significant progress in food and feed safety. This allows growers to harvest infected and healthy grain separately, avoiding the risk of mixing contaminated and non-infected grain for storage. Separate harvests are also advisable because *Fusarium* fungi proliferation and mycotoxin synthesis can occur in storage under certain conditions. In addition, the risk of food mycotoxin poisoning can be reduced and the rational use of *Fusarium* infected grain can be facilitated.

Throughout the cereal production chain, there are a variety of options and management practices to prevent Fusarium infection and, thus, head blight (Figure 1). Firstly, Fusarium tolerant wheat varieties should be selected for cultivation. In crop rotation, cereals such as maize, wheat, durum barley, which, if possible, spread Fusarium easily. Furthermore, tillage can destroy infected grain stalks or straws, which can serve as inoculum the following year. It also prevents the hibernation of fungal spores and therefore faster distribution the following spring. If disease pressure is high, eg, due to adverse weather, fungicides should be applied shortly before flowering. At this time, preventive spraying of azole is done over the entire field to prevent Fusarium infection. Undoubtedly, carefully targeted application of fungicides can be economically and environmentally beneficial. However, this routinely requires accurate knowledge of the true, site-specific status of preharvest Fusarium infection.

Automated reliable Fusarium detection is urgently needed because the frequency of infection is increasing, and as a result, legal provisions have been intensified. Manufacturers are waiting for innovative detection methods and equipment. Although not currently applied by default in crop production, such knowledge can still be obtained through field monitoring with many recent imaging techniques. This review introduces both chlorophyll fluorescence (CFI) and hyperspectral imaging as means for rapid site-specific on-field detection of head blight. It also presents recent advances in these techniques and discusses their potential and limitations for practical applications of these methods.

DETECTION OF HEAD BLIGHT SYMPTOMS

During initial successful infection, Fusarium induces various internal changes and host-specific responses in inoculated plants. As a result, the symptoms of the disease usually do not appear immediately, but later appear externally. 6-10 days after vaccination. Only then, these symptoms can be detected and analysed with Spectro-optical reflectance measurements in the visible (VIS) and near-infrared (NIR) range but also with fluorescence spectroscopy. External, and to some extent cuticle, also host internal biochemical changes of cell-walls, epidermis cells, etc. Changes can be evaluated by fluorescence and NIR measurements. In addition, fungal effects on specific tissue properties, such as composition and total content of leaf pigments or changes in cell water, sugar or protein content, can be investigated by means of emission measurements in the VIS and NIR ranges, respectively. On the other hand, in a progressive infection, the effect of Fusarium on the metabolic capacity of the host at the cellular level does not necessarily lead to the development of outward symptoms. These plant responses can be comprehensively monitored by analyzing chlorophyll fluorescence transients, which, among others, indicate the integrity of the photosynthetic apparatus.

Evolution of Fungal Infection by Chlorophyll Fluorescence Spectral:

Chlorophyll fluorescence analyzes are well-established, effective tools for the comprehensive investigation of the development and effects of bacterial, fungal, and viral infections on leaves of many cultivated plants. It can be used for whole intact plants, detached leaves and leaf discs extracted from infected plant material. CFI was applied to wheat, among others, to determine the effects of drought and heat stress, limited supply of nutrients and various diseases such as leaf rust, leaf and glume blotch or powdery mildew. For disease detection, the empirical fluorescence parameter F_v/F_0 is proposed for use on dark adapted plants. Although a clear physical derivation is still lacking, F_v/F_0 presumably represents the maximum quantum yield of fluorescence. This parameter has been used as an indicator of photosystem II (PSII) status and can predict rates of energy transport from PSII to PSI in low-temperature fluorescence.

In addition, the potential maximum quantum yield of electron flow through fully open PSII, F_v/F_m , is often used to assess microbial diseases. F_v/F_m reflects maximum photochemical efficiency and interference from various environmental factors and may also indicate potential pathogen-related functional disruption of the photosynthetic apparatus. Fusarium infections have immediate effects on F_v/F_m because the fungus rapidly and strongly impairs metabolism and, thus, photosynthetic processes of infected spikelets or head parts of host plants. Mycotoxins produced by fungi can also cause a complete reduction in photosynthetic efficiency, as observed in maize and banana infections by *Colletotrichum muae* and *Fusarium moniliforme*. Therefore, it has been observed that the reduction of

F_v/F_m , and F_v/F_0 is also closely related to the degree of infection and, therefore, is a suitable parameter for the detection of headache and other fungal diseases.

Detection of Time Frame:

Because it closely reflects the physiology of photosynthesis, chlorophyll fluorescence imaging enables early detection of Fusarium infection-related tissue damage. Indeed, it has been demonstrated that differences in photosynthetic activity and chlorophyll fluorescence patterns can be detected at early stages of infection. However, the earliest changes at the cellular level had only minor effects on PSI. Only when the integrity of the cellular structures of the host plants was damaged by the fungus did the photosynthetic system become impaired. This explanation reflects light and electron microscopy findings, and these authors showed that dominant fungal hyphae cause pronounced cellular changes only after infection, after 5 to 6 days.

The ability of chlorophyll fluorescence analysis and imaging, respectively, to detect fungal infection does not depend solely on the time period after inoculation; It also has a lot of influence on the object to examine itself. *Pseudomonas syringae* infection in *Arabidopsis thaliana* can be detected within hours after inoculation. In contrast, F_v/F_0 decreased two to three days before leaf rust and fungal infection were observed on winter wheat leaves, respectively. Pustules of leaf rust appeared 6 days after inoculation (Die), while blight symptoms usually appeared from the 9th day. With leaf rust infection, F_v/F_0 significantly decreased by about 0.4 relative units after the 6th day, while the decrease in F_v/F_m was much smaller (0.02 relative units) compared to uninfected controls. The first symptoms of *Venturia inaequalis* infection on apple plants can be observed from the 7th. In common spruce seedlings, needle rust infection can be detected by CFA three weeks after infection but not at an early stage.

The time period for meaningful detection of Fusarium infection of wheat with CFA and CFI is also limited. With the onset of wheat head maturation, the chlorophyll content of spikelets inevitably decreases and F_v/F_m also decreases, regardless of infection or not. As a result, relative cumulative F_v/F_m increases rapidly in low F_v/F_m classes. Logically, this parameter is not suitable for detection of biotic disease on fully mature wheat heads after reaching the final grain development stage.

Finding the accuracy of CFI on wheat plants with different degrees of Fusarium infection:

Potential maximum photochemical efficiency F_v/F_m easily indicates photosynthetic machinery damage by *Fusarium culmorum*. A very high detection accuracy (15% RMSE) can be achieved using relative cumulative F_v/F_m (rcF_v/F_m) at a threshold of 0.3. The relative cumulative F_v/F_m values in the low performance class gradually increased from <0.1% to 3%–4% Fusarium infection (degree of infection, doi) to 15% at doi of 15%–25%, holding approximately 45%–65%. It reaches 85% at infection rates of 35% and at infection rates of 90%. In the above investigation, the average F_v/F_m over the heads analyzed determined all these differences. However, the use of different F_v/F_m value classes accurately observed the detrimental effects of Fusarium on photosynthesis. Beyond a minimum doi of 5%, the relative degree of transition can be easily identified in steps of 15% with this method. In other investigations, where all efficiency classes were averaged, changes in F_v/F_m with progressive infection remained small and could only be inadequately resolved.

Field Condition Application:

The application of CFI to the detection of fungal diseases under field conditions was occasionally tested. For this purpose, a 10-bit camera with a resolution of 1350 pixels × 1050 pixels, a four-band optical beam splitter, a pass-band filter, a xenon arc lamp with an IR cut-off filter, and a low-pass filter, were used. Using a chlorophyll fluorescence imaging system patented. the recording of steady-state fluorescence signals is definitely easier than the fluorescence kinetics □PSII conditions as a "very useful parameter" to measure CF under real environmental conditions. Undoubtedly, this parameter does not require pre-darkening of the measured object. However, □PSII strongly depends on highly variable prevailing daylight conditions, and therefore, should be related to specific standards. Due to their complex physiological nature, fluorescence signals are directly dependent on photosynthetic photon flow rates. In addition, the values of the light-adapted steady state fluorescence signal (F_0' , F_m') are

lower than those of the dark-adapted (F_0 , F_m) and the measurement changes caused by fungal diseases can be detected less clearly.

Current techniques of chlorophyll fluorescence imaging, such as the modular system of the FluorCam MF700, used under field conditions to detect head blight, certainly require adaptation for this specific application. Furthermore, it is necessary to change the photon fluence rate during measurement and avoid direct exposure to sunlight, while recording F_0 and F_m requires dark adaptation of plants. If these requirements are met, CFI can be successfully applied in outdoor situations.

Systematic problems in outdoor measurements with current CF imaging systems can be minimized with proper use. CFI-scanning is especially difficult in windy conditions. Due to the movements of the wheat head during the recording of the F_0 and F_m sequences, the images of these parameters may not completely overlap. Reducing the total recording time to 2s can greatly reduce this problem. Nevertheless, the peripheral region of the head, most affected by wind, may show artificially low F_v/F_m values and consequently the marginal region of the ROI must be excluded from further analysis. Also, incomplete or uneven shading can lead to overestimation of basal fluorescence and, as a result, falsely low F_v . Therefore, for outdoor use, measurement techniques and protocols and algorithms for elimination of outliers need to be adapted to take advantage of the high potential of CFI to detect non-invasive disease.

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