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ARTICLE

Characterization and early detection of tan spot disease in wheat *in vivo* with chlorophyll fluorescence imaging

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ABSTRACT Plant growth is affected by various factors. The resistance of the plant to withstand various biotic and abiotic stress factors plays a vital role for its growth and development. In this study, we have characterized the resistance of various wheat cultivars to tan spot disease by the application of imaging techniques (such as: digital photography, chlorophyll fluorescence) under controlled conditions. The increase of the F0 chlorophyll fluorescence indicates the production of free chlorophylls, which may be involved in the defense reaction against the pathogen. The fluorescence data revealed changes of the photosynthetic apparatus at an early stage before the appearance of visual symptoms appeared, which could be used as an easily measurable markers of the infection.
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KEY WORDS

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fluorescence imaging
PAM fluorometry
photosynthetic Apparatus

Tan spot disease caused by the fungal pathogen *Pyrenophora tritici-repentis* (PTR) represents a significant threat to wheat production. The sensitivity of wheat to PTR varies in a wide range from very high susceptibility to complete, symptomless resistance. However, the molecular background of resistance is often poorly understood.

The aim of the present work was to develop sensitive non-invasive imaging methods, which makes possible the quantitative characterization of leaf infections. Further aim of the work was the characterization of various Hungarian and Romanian wheat cultivars regarding their sensitivity against the *PTR* pathogen, as well as to obtain a better understanding of the mechanisms, which are involved in the infection and the various levels of tolerance in the different cultivars.

Materials and Methods

We have developed a method to assess visual symptoms of the infection by digital color (RGB) imaging in combination with variable chlorophyll fluorescence imaging to assess the photosynthetic activity of the infected areas. The RGB imaging was performed with a high resolution digital camera (Nikon D70), whereas chlorophyll fluorescence imaging was performed by a fluorescence camera system (WALZ Fluorcam). We have also developed mathematical tools and software for the analysis of the digital RGB and fluorescence images.

We have used 2 Hungarian and 2 Romanian wheat cultivars currently used in production and differing for tan spot resistance, as well as 3 standard lines/cultivars (M3, ND495 and Alcedo) for DTR infection (Table 1.). Selection of cultivars was based on previous tests for tan spot resistance in adult stage (Csősz, unpublished results).

Wheat plants were grown in greenhouse (20°C day/ 15°C night temperature with 12 hours/day illumination) were inoculated at the age of 14 days by a *PTR* monospore isolate. After inoculation plants were covered with plastic bags for 2 days to provide 100% air humidity. From each cultivar 3-4 leaves were selected for the measurements. Selected leaf areas were monitored for the development of infections, and assessed by changes in the RGB and chlorophyll fluorescence images. RGB and fluorescence changes, which became clearly visible in the later phases of the infections, were traced back on the same leaf area at the early stages of the infections.

Results

Visual symptoms of infection

Tan spots were almost completely absent in the standard line M3 and Hungarian cultivar GK Héja even after 21 days.

Table 1. Data of wheat genotypes used in investigations.

Cultivar	Origin	Resistance to DTR
Alcedo	Germany	Moderately susceptible
GK Verecke	Szeged, Hungary	Susceptible
GK Héja	Szeged, Hungary	Resistant
M3	USA	Very resistant
ND495	USA	Very susceptible
Lovrin 34	Lovrin, Romania	Moderately susceptible
Alex	Lovrin, Romania	Moderately resistant

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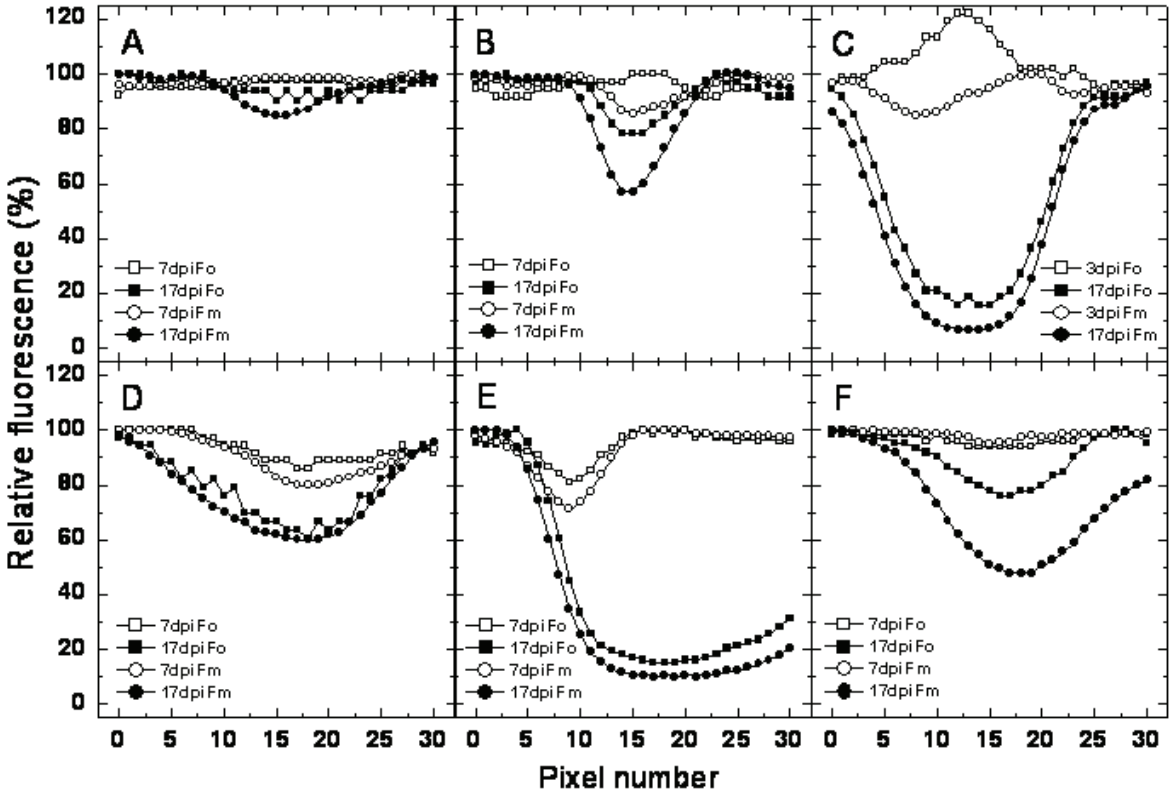

Figure 1. Changes of the F0 and Fm fluorescence parameters via transect lines cutting through the infected area during development of leaf spot disease in different wheat varieties. A: M3, B: GK Héja, C: Alcedo, D: GK Verecke, E: Alex, F: Lovrin 34.

In case of moderately susceptible standard cultivar Alcedo few spots appeared, but they were localized to a small area relative to the total leaf surface. The susceptible Hungarian cultivar GK Verecke had only few spots until 15-17 days, which were localized on a relatively small area, but eventually developed large areas of infection between days 17-21. The very susceptible standard line ND495 had only few, but not well localized spots until day 11, but developed large areas of infection between days 11-21. Similar behavior was observed in both Romanian cultivars, in the moderately susceptible Lovrin 34 and the moderately resistant Alex.

Fluorescence characteristics of the infection

The Fv/Fm fluorescence parameter, which reflects the efficiency of Photosystem II electron transport was unaffected up to 17 days in M3, GK Héja and Alcedo, and showed slight decrease between days 19-21. In case of cultivar GK Verecke, Fv/Fm dropped significantly after day 17, but keeps reasonable activity until day 21. In case of ND495, Lovrin 34 & Alex, the loss of Fv/Fm starts already after days 11-15 and shows an almost complete inhibition of photosynthetic activity over the whole leaf area.

We have also analyzed the kinetics of the development of leaf infection by measuring the F0 and Fm values along transect lines, which cut through the infected areas. In case of the strongly resistant line M3, the loss of F0 and Fm is very small in the area of the few detectable spots. F0 and Fm is affected to similar extent, which indicates that the loss of chlorophyll and of photosynthetic activity are correlated. The extent of the infection developed slowly and remained at a low level (Fig. 1A). In case of the also strongly resistant GK Héja, there were only few spots of infection. However, in contrast to line M3, the F0 and Fm values were changing in an opposite way in the early phase of the infection. Up to 8-9 days F0 was increasing, while Fm was decreasing in the area of the infection, although visual symptoms were still absent. In the later phase of the infection not only Fm, but also F0 decreased, but the extent of the F0 loss remained always significantly smaller than that of Fm (Fig. 1B). This shows that the loss of photosynthetic activity precedes the loss of chlorophyll amount. In case of standard cultivar Alcedo, which showed moderately susceptible reaction to PTR, but also developed well localized spots, the F0 value increased while Fm was unaffected or decreased at the beginning of the infection when visual symptoms could not be detected yet.

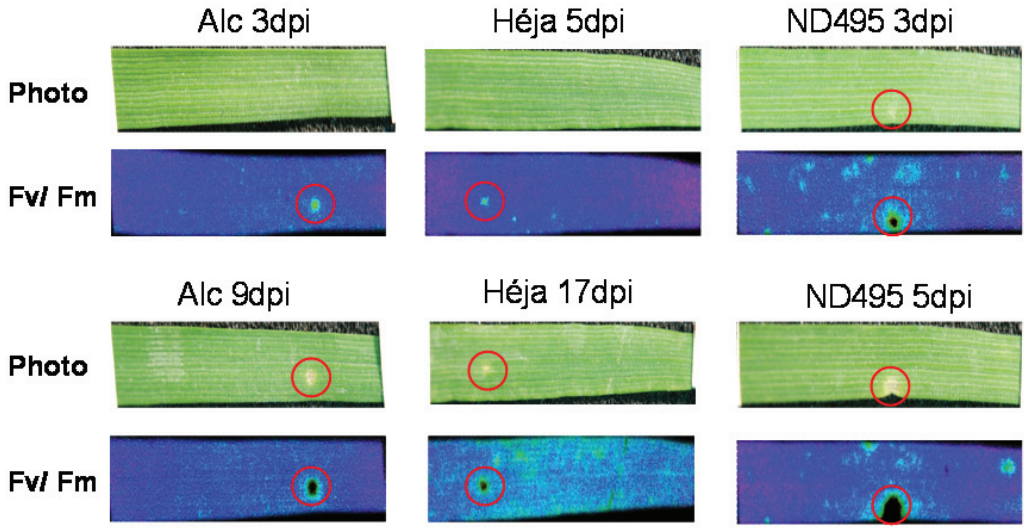

Figure 2. Early warning fluorescence changes during leaf spot disease in different wheat cultivars.

This situation was similar to that observed in GK Héja, but lasts for a shorter period.

In the later phase of the infection the change of both F0 and Fm showed a symmetric bell shaped distribution whose amplitude and width gradually increased with time after the infection. However, the infection remained localized (Fig. 1C). Within the spots the extent of Fm loss is always higher than the loss of F0.

In case of the GK Verecke and ND495 cultivars, which showed strong and very strong sensitivity, respectively the loss of F0 and Fm started to develop 6-7 days after the infection. The size of the affected area, as well as the extent of F0 and Fm loss gradually increased with time after the infection. The extent of F0 and Fm loss were similar (Fig. 1D), which shows correlated loss of chlorophyll and photosynthetic activity. These fluorescence features are similar to those observed in M3.

In cultivars Alex and Lovrin 34 which showed symptoms from medium susceptibility to medium resistance, repectively, the extent of F0 loss was smaller than that of Fm (Figs. 1E, F), which shows that PSII activity is damaged faster than chlorophyll content. These fluorescence features are similar to those observed in GK Héja, and Alcedo.

Early detection of PTR infection by chlorophyll fluorescence

An important aim of the work was to clarify whether or not fluorescence changes can be used for detection of the infection before visual symptoms appear. In cultivar GK Héja, this seems to be the case since F0 and Fm changes appeared 3-4 days before the visual symptoms (Fig. 2). However, the effect was not very characteristic due to the overall high degree of resistance of cultivar GK Héja. The change of F0 and Fm appeared also earlier in cultivar Alcedo than the visual signs of the infection. The effect was more pronounced than in GK Héja, but lasted for a shorter period (1 day). In other cultivars the appearance of the visual and fluorescence changes were more or less parallel.

Conclusions

Our study shows that chlorophyll fluorescence imaging is a useful method for the detection of characterization of tan spot infection in wheat. We could identify three groups among the wheat varieties studied with different level of resistance against PTR infection, as well as two groups regarding putative resistance mechanisms. The three groups which differed in the extent of resistance were the following: (i) cultivar GK Héja and standard line M3 showed significant resistance and survived the exposure to the pathogen with very few visual symptoms, and kept photosynthetic activity intact. (ii) Cultivar Alcedo also showed a large extent of resistance as regards the limited infected area and high overall photosynthetic activity. Nevertheless, it developed few infection spots, which remain well localized. (iii) Genotypes GK Verecke, Alex, Lovrin 34 and ND495 developed relatively small areas of infection in the first phase of infection, but later the infection was delocalized rapidly and destroyed large areas.

The two groups, which could be identified on the basis of possibly different mechanisms of resistance are: Group-I (GK Héja, and M3) share the common feature of having larger extent of Fm loss as compared to F0, as well as inducing high F0 in the early phase of infection (GK Héja and Alcedo). This feature indicates that free chlorophylls are probably produced as an early response to the infection. The smaller extent of

F0 loss as compared to Fm in the later phase of the infection can also arise from the presence of free chlorophylls, which are not connected to the photosynthetic apparatus and partly compensate the overall loss of chlorophylls. Free chlorophylls are known to sensitize singlet oxygen production, which can participate in the defense against the pathogen attack by inducing cell death. Group-II (GK Verecke, Alex, Lovrin 34 and ND495) cultivars share the common feature of losing F0 and Fm to similar extent during the progress of the infection. This shows that free chlorophyll does not accumulate, and probably not involved in the defense reaction.

It is also of note that in cultivars GK Héja and Alcedo the change in the fluorescence parameters makes possible the early detection of infection few days before the appearance of visual symptoms. However, in the other cultivars the fluorescence and color changes appear in parallel, which does not make possible the early detection of infection on the basis of fluorescence parameters.

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