# SUITABILITY OF FLUORESCENCE SENSORS TO ESTIMATE THE SUSCEPTIBILITY DEGREE OF SPRING BARLEY TO POWDERY MILDEW AND LEAF RUST

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## ABSTRACT

The target-oriented selection of stress-tolerant genotypes and its contribution for a more sustainable agriculture is receiving increased attention, also in the scope of precision agriculture. The selection of genotypes in breeding programs usually targets the increase of yield under consideration of production factors, and is done in the field by breeders and trained evaluators. Particularly in extensive breeding programs at advanced stages the evaluation, categorization and selection of promising and more adequate genotypes impose a big challenge even to experienced workers. Thus, the development and optimization of objective tools for fast and reliable data recording is essential to better estimate the potential and precisely differentiate genotypes in highly homogeneous populations. In plant sciences, the fluorescence spectroscopy is an established approach for basic and applied research. In agronomy and precision farming its potential is already confirmed e.g., for the site-specific nitrogen fertilization. Moreover, some studies suggest its potential for sensing of foliar diseases and other environmental stress. However, commercially available systems have very different technical specifications, not only related to the fluorescence excitation and recording (e.g., intensity and quality of excitation light, detection wavelengths, temporal resolution, camera-based recordings), but also to their robustness and suitability for the use in the field. Therefore, the quality and usefulness of the information provided by different systems might vary considerably.

In our work we explored the relation between the pathogen-induced alteration of characteristic fluorescence signals of four barley cultivars (Belana, Marthe, Conchita, Tocada) and their susceptibility degree (SD) to leaf rust (*Puccinia hordei*) and powdery mildew (*Blumeria graminis*). The susceptibility degree of the selected cultivars (classified by the German Federal Plant Variety Office, 2010 in a scheme from 1 (low) to 9 (high) susceptibility) ranged from 2 to 7 for powdery mildew, and 4-6 for leaf rust. The experimental plants were inoculated with either powdery mildew or leaf rust, and fluorescence recordings were taken at leaf level at three, six and nine days after inoculation (DAI). With a laboratory laser fluoroscope (IOM® Lambda 401, Berlin, Germany) we recorded the time-resolved fluorescence mean lifetime (LTmean) at six previously defined

wavelengths (410, 440, 470, 500, 530, 560 nm); with a multispectral fluorescence camera (Nuance® CRI, Perkin-Elmer, USA) we recorded the fluorescence intensity (420-720 nm) with spatial resolution after UV, blue and/or green excitation, and calculated the area affected by pathogens; with a portable hand-held multiparametric fluorescence sensor (Multiplex®, Force-A, France) we recorded the fluorescence intensity in the blue, red, and far-red spectral bands after UV, green and/or red excitation, and used the signals to calculate specific fluorescence indices. In all cases, non-inoculated leaves served as control.

Evaluations at 3 DAI reveal a slight increase of the LTmean at specific wavelengths, but there was no clear relation with the SD to powdery mildew. At 9 DAI the cultivars Belana and Tocada (higher SD) had significantly higher LTmean in all wavelengths, while the cultivars Marthe and Conchita showed no significant differences, as compared to the respective controls. Differently, leaves inoculated with leaf rust had higher LTmean than control plants in several wavelengths at 3 DAI, and in all wavelengths at 9 DAI, irrespective of the SD (which ranged only from SD = 4 to SD = 6). With the spectrally-resolved fluorescence images we confirm the stronger impact of pathogen infection in the blue fluorescence (420-500 nm; excited with UV) and green fluorescence (500-580 nm, with blue light). In particular, the inoculation with powdery mildew was followed by a rapid increase in the affected leaf area (calculated from the fluorescence pictures) and the respective green fluorescence intensity in all cultivars. Differently, rust inoculated leaves showed an increase of the affected area over the time, while the intensity of green fluorescence decreased in inoculated leaves. These results confirm our previous observations and are strongly related to the green fluorescence emitted by the structures of the fungi.

With the hand-held multiparametric fluorescence sensor, we focused on two indices, the ‘Blue-to-Far-red fluorescence ratio’ (BFRR_UV) and the ‘Simple Fluorescence Ratio’ (SFR). Particularly in the cultivars susceptible to powdery mildew, BFRR had a stronger increase after inoculation as compared to the less susceptible ones. Moreover, the SFR, a ratio which depends on the intensity of the chlorophyll fluorescence, reveals a dynamic process characterized by increase of values in diseased leaves at 3 DAI followed by a decrease to 6 and 9 DAI.

In summary, we show that a) the fluorescence-based sensing of leaf rust and powdery mildew can be accomplished with selected fluorescence parameters already at 3 days after inoculation; b) the impact of the pathogens in changing the fluorescence signature becomes stronger in the time course of the experiments; and c) genotype-specific alterations of the fluorescence signature due to the occurrence of diseases might occur, but are not necessarily related to the susceptibility degree of the plant. Nevertheless, our results point to selected fluorescence indices for early sensing of diseases under laboratory and field conditions. Some of these selected indices might potentially be used as additional evaluation tool in plant breeding and supportive activities in precision farming.

**Keywords:** Biotic stresses, detection, differentiation, fluorescence ratios

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