Chlorophyll fluorescence as a plant stress indicator

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The objective of the work is to determine the degree of plant stress detectable from fluorescence measurements. Experiments have been conducted with Hordeum vulgare grown in greenhouse conditions for 2–3 weeks. Plant stress in response to the application of different concentrations of Cd has been quantified by changes of leaf pigment content. Heavy metals affect plant photosynthetic apparatus causing. Fluorescence spectra exited at 470 nm have been taken in the red and far-red spectral region (640-800 nm). Significant increase of fluorescence emission of stressed plants in comparison to control plants has been observed. The variance of different fluorescence parameters has been statistically related to chlorophyll decrease. The results show that the heavy metal induced stress is detectable from chlorophyll fluorescence demonstrating that the analysis of fluorescence spectra may timely and accurately indicate the onset of stress in plants.

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Introduction

Much interest in the early assessment of growth stress of agricultural crops has been generated recently by the spreading acceptance of the precision agriculture running concept. The implementation of modern remote sensing technologies is a basic assumption of this concept [1]. That is why the assessment from spectral data of crop state and growing conditions is in the focus of many investigations and experimental studies [2-4]. Special attention is paid to vegetation monitoring in relation to stress detection [5-8].

Stress-induced changes in the composition of photosynthetic pigments change the optical signatures of leaves and as such are indicative of plant functioning and short-term or long-term stresses. The early detection of plant inhibited growth should be directly connected to fundamental processes of plant physiology. Such a fundamental process is photosynthesis and the connection has been found in vegetation fluorescence. Chlorophyll fluorescence is a measure of the efficiency of photosynthesis and can be used, therefore, as an indicator of vegetation health and vitality. Though being studied for decades, light induced fluorescence has not lost its attractivity to experimental plant physiology. Moreover, it experiences ever-increasing interest as a response to different stresses that might be qualified and quantified from plant fluorescence behaviour. In recent years, the screening of plant fluorescence signatures is developing as a specific tool which could be applied to detect the functioning and health status of plants [7,9,11]. Compared to reflectance, induced fluorescence might be a more accurate indicator of plant state and be able to detect stress impacts at earlier growth stages.

The objective of this paper was to examine the relationship between fluorescence emission spectrum and barley growth inhibition under stress conditions (heavy metal contamination). Fluorescence changes appeared to be high-sensitive to chlorophyll decrease at a very early stage of plant development before visual color or morphological signs had been observed.

Materials and Methods

In the experiment barley plants (*Hordeum vulgare* L., standard karyotype) were used. Seeds were imbibed in tap water for 1 h at 27°C and germinated for 48 hrs between two layers of wet filter paper at 27°C in darkness. The seedlings were planted on perforated polystyrene plates, floating on distilled water in glass pots, and were grown during 5 days under day/night environmental conditions. Then the plants

were transferred to glass pots with distilled water (V=350 ml/pot) supplemented with stock solution of $CdCl_2$ so as to give 10, 20 and 30 mg/l heavy metal concentrations in the medium. After that the plants were grown for 8-9 days.

The fluorescence measurements were performed using the developed in STIL-BAS spectrometric system SPS-1 [10] with an excitation wavelength 470 nm, light intensity 507μmol.m^{-2}.s^{-1} and exposure time of the photodetector fixed at 1.5 s. Each record consisted of 10 consecutive spectra, automatically averaged. The fluorescence was obtained from the upper side of leaf samples after 3 min predarkening. The records were corrected for the spectral sensitivity of the measurement device.

The degree of stress was quantified through changes in leaf pigments since all plant stressors either directly or indirectly affect the photosynthetic apparatus and its function causing a decline of the chlorophyll content. On the same day as the fluorescence measurements were made, plant chlorophyll *a*, chlorophyll *b* and carotenoid concentrations were determined spectrophotometrically by grounding 100 mg fresh weight of leaf material in 10 ml of 80% (v/v) acetone at 4°C. Pigments' content was calculated by Arnon (1949).

For data processing the following fluorescence parameters were determined: fluorescence intensities of the red F690 and far-red F740 emission maxima, fluorescence minimum F710 between these two bands, the corresponding wavelengths. Besides, various ratios and contrasts between fluorescence intensities were calculated as well as the bandwidth between both emission peaks. These fluorescence features and plant chlorophyll values were statistically analyzed in order to quantitatively examine their relationship. Correlation and regression analyses were performed. The results were tested by independent data sets from repeated experiments.

Results and Discussion

Visible light illumination excites chlorophyll fluorescence of high intensity in the red and far red spectral band (675-740 nm) with peaks at about 685-690 nm and 735-740 nm [7]. Fluorescence spectral shape of leaves is sensitive to chlorophyll content [9,11]. This fact is very important, since many kinds of plant damage lead to a reduction of leaf chlorophyll. Typical fluorescence spectra obtained in our experiments from barley leaf samples are shown in Figure 1. They illustrate the effect of different chlorophyll concentration on the parameters of the recorded fluorescence signal.

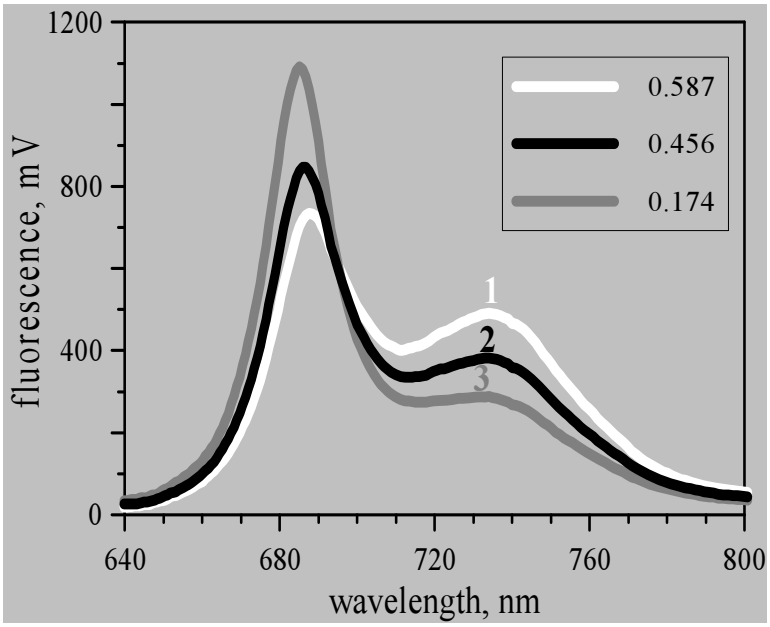

Figure 1. Red and far-red fluorescence of barley leaves with different chlorophyll concentration (see Table 1)

Table 1. Fluorescence parameters and chlorophyll concentration of barley leaves from Fig.1

Fluorescence parameter	***Chlorophyll concentration [mg/g]***		
	0.587	0.456	0.174
max F690 [mV]	725	850	1100
min F710 [mV]	425	350	260
max F740 [mV]	510	400	320
λF690 [nm]	687.52	686.71	685.11
λF710 [nm]	717.1	714.7	713.3
λF740 [nm]	733.98	733.98	733.98
Δλ(F740-F690)	46.46	47.27	48.87

Changes of the fluorescence peak intensities (termed as F690 and F740 – Figures 2a and 2b) were observed as well as of the minimum emission values at about 710 nm. Plants with higher degree of stress exhibited increased red fluorescence and decreased far-red intensity along with decreased F710 minimum. Furthermore, the wavelength of the red fluorescence F690 peak manifested shifts towards longer wavelengths with increasing chlorophyll content while the position of the far-red maximum remained consistent. Small wavelength shifts were observed also in the F710 emission minimum but with the opposite to λF690 behaviour, i.e. to shorter wavelengths with increasing chlorophyll content. All these changes are due to varying chlorophyll and as such chlorophyll changes are readily traceable through fluorescence measurements. In Table 1 the fluorescence features of barley leaves corresponding to Figure 1 are presented. They show the measured intensities of the red F690 and far-red F740 fluorescence maxima and F710 minimum, their centre wavelengths and the bandwidth between both emission maxima.

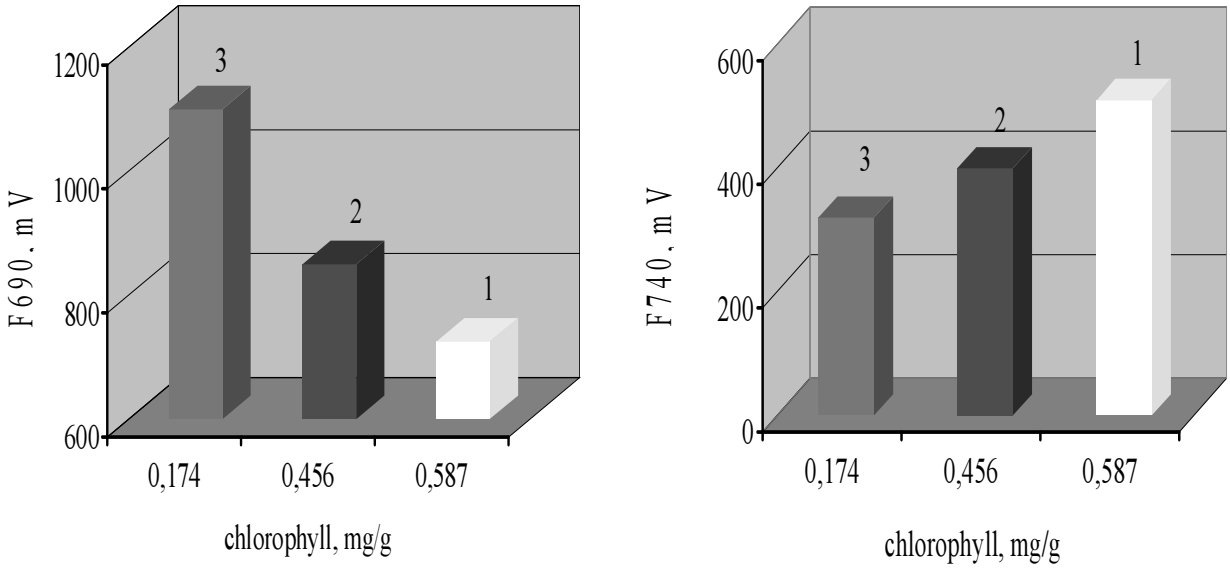

Figure 2. Fluorescence peak intensities at F690 (a) and F740 (b) with different chlorophyll concentration (see Table 1)

In Table 2 results of the linear correlation analysis between barley fluorescence and chlorophyll concentrations data sets are given (about 50 measurements from one experiment). As commented above, the correlation of the chlorophyll with the F710 and F740 intensities is positive and the correlation with the F690 emission and the wavelength width Δλ=λF740-λF690 is negative. Vigorous vegetation manifested low fluorescence emission in the red spectral band. The width between the two fluorescence

peaks appeared to be most indicative of chlorophyll changes. Plant stress defined in terms of chlorophyll reduction increases the fluorescence emission width.

Table 2. Coefficients of linear correlation between chlorophyll concentration and fluorescence features of barley leaves

Fluorescence parameter	***F690***	***F710***	***F740***	***λF740-λF690***
Coefficient of linear correlation	-0.69	0.49	0.68	-0.86

High sensitivity to chlorophyll, however, revealed the relative intensities of the red and far-red fluorescent bands. Fluorescence response in terms of F690 and F740 was exponentially related to leaf chlorophyll. The established empirical relationship between fluorescence at F690/F740 ratio and barley chlorophyll concentration is presented in Figure 3a. Besides the high correlation (R^2=0.88 at $p<0.001$), the adequacy of the fitted model was tested and confirmed by an independent data set from repeated experiments.

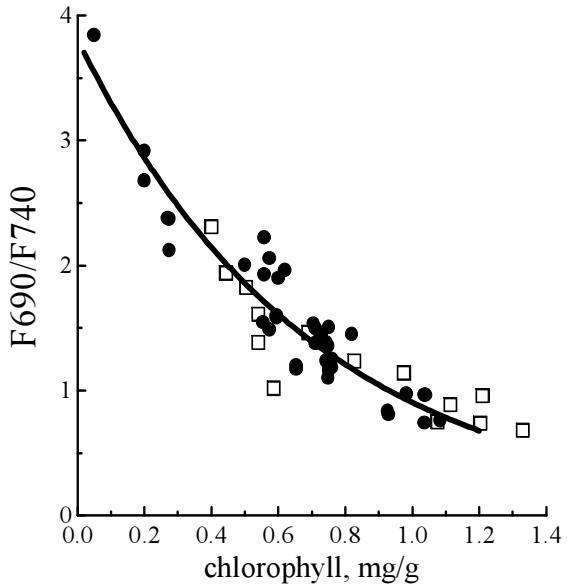

Figure 3a. Regression (R^2=0.88) between chlorophyll concentration of barley leaves and F690/F740 intensity ratio (●); independent data from repeated experiments (□)

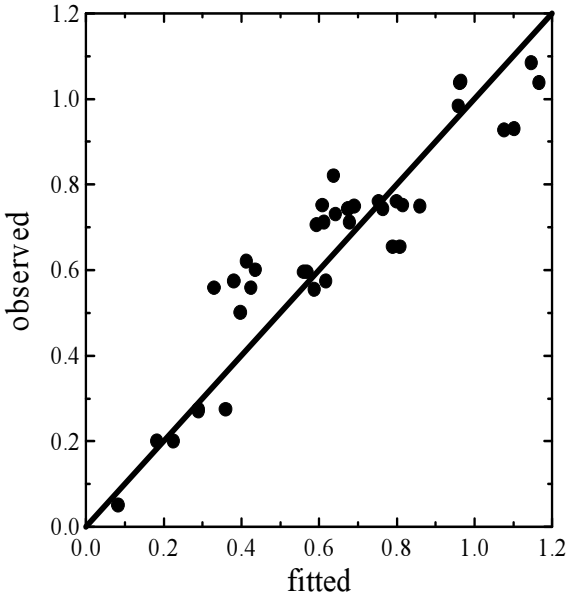

Figure 3b. Correspondence between measured and fitted through F690/F740 chlorophyll values of barley leaves

Figure 3b shows the correspondence between the observed (measured) and fitted (modeled) chlorophyll values. The predicted chlorophyll concentration was in good agreement with the experimental data.

The differences of the fluorescence intensities were quantified by forming other fluorescence ratios as well (similar to reflectance vegetation indices). They were exponentially regressed to chlorophyll data and most of them showed significant correlation with leaf chlorophyll content. Some of the results are presented in Table 3.

Table 3. Parameters of exponential regressions of chlorophyll concentration [mg/g] of barley leaves on fluorescence intensity ratios

Fluorescence parameter	***Regression coefficients***		***R^2***
	a	***b***	
F690/F740	0.819	-0.851	0.88
(F690-F740)/F740	-0.025	-0.848	0.87
F690/F710	1.052	-0.835	0.84
(F690-F710)/F710	0.218	-0.835	0.84
(F690-F710)/F740	0.163	-0.927	0.83
(F690-F740)/F710	0.001	-0.784	0.85

Conclusions

The present experimental study had been designed and accomplished with the goal of getting information upon the sensitivity of fluorescence measurements to variations of leaf chlorophyll content and the possibility to assess plant stress by quantifying this dependence. Convincing results were obtained validating the fluorescence signal as a valuable source of information and an efficient tool for vegetation state assessment. They can be summarized as follows.

Relative to control and less stressed barley plants there was an increase of the fluorescence emission intensity of stress-suffering plants across the whole red and far-red spectral range. The experiments showed that heavy metal induced stress were detectable with measurements of fluorescence spectra revealing significant changes in fluorescence shape (intensity, wavelength shifts, and bandwidth). Various fluorescence ratios appeared to be closely related to chlorophyll content allowing the establishment of quantitative dependences and proving useful for the detection of decreases in leaf chlorophyll content.

The chlorophyll fluorescence spectrum of leaves depends also on the wavelength of the excitation light. Blue light excited fluorescence exhibited high emission values which make the red and far-red fluorescence parameters more sensitive to small chlorophyll changes in plant leaves.

Because of other factors affecting the emission signal (leaf roughness, thickness) fluorescence absolute values can vary from sample to sample. Too much smaller degree varies fluorescence intensity ratios. That is why fluorescence intensities alone are not as reliable as fluorescence ratios which exhibit a much lower variation from leaf to leaf and represent reliable and reproducible means for quantification of plant state assessment.

An important aspect was the fact that fluorescence parameters manifested themselves as very early indicators of stress events (during the second-leaf stage of barley plants). Thus fluorescence measurements proved as an indicator of chlorophyll content and a reliable tool for early detection of plant inhibition as it is demonstrated here.

These results show that the analysis of fluorescence emission spectra may accurately indicate the onset of stress in plants. Even performed on leaf level fluorescence measurements might be useful for physiological control of agricultural crops and for detecting plant stress in terms of chlorophyll inhibition.

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