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# Laser-induced chlorophyll fluorescence and reflectance spectroscopy of cadmium treated *Triticum aestivum* L. plants

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**Abstract.** The present study deals with laser-induced chlorophyll fluorescence (LICF) spectra, reflectance spectra and fluorescence induction kinetics (FIK) curves of *Triticum aestivum* L. plants treated with different concentrations of cadmium (0.01, 0.1 and 1.0 mM). LICF spectra were recorded in the region of 650–780 nm using violet diode laser (405 nm) and FIK curves were recorded at 685 and 730 nm using red diode laser (635 nm) for excitation. Reflectance spectra were recorded in the region of 400–800 nm using spectrophotometer with an integrating sphere. The fluorescence intensity ratios (FIR) were determined from LICF spectra, vitality index ($R_{fd}$) from FIK curves and narrow band vegetation index (NBVI) from reflectance spectra. These parameters along with plant growth parameters and photosynthetic pigment contents were used to analyze the effect of cadmium on wheat plants. The results clearly show that lower concentration of Cd (0.01 mM) shows stimulatory response; whereas higher concentrations (0.1 and 1.0 Mm) are hazardous for plant growth, photosynthetic pigments and photosynthetic activity of wheat plants.

Keywords: Laser-induced chlorophyll fluorescence, fluorescence induction kinetics, reflectance spectra, photosynthetic pigment contents, wheat plants, cadmium stress

## 1. Introduction

Laser-induced chlorophyll fluorescence (LICF) and reflectance measurements have widespread application in field studies and remote assessment for detecting environmental stress induced physiological changes in leaves of plants. The study of laser-induced *in vivo* chlorophyll (Chl) fluorescence of green plant leaves provides basic information about the functioning of the photosynthetic apparatus and also regarding the capacity and performance of photosynthesis. A large part of the visible light absorbed by the photosynthetic pigments is used for photochemical conversion in photosynthesis. However, a small proportion of the absorbed light energy is re-emitted either as heat [10], or as red fluorescence, RF [25]. Under various stress conditions, the rate of photosynthesis ($CO_2$-assimilation) is considerably reduced due to disruption of the light-driven photosynthetic electron transport, the pigment apparatus and/or the

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$CO_2$ assimilation, without affecting the process of light absorption. This then leads to an increased de-excitation of the absorbed light via Chl fluorescence. It was first observed and described by Kautsky [16], and it can be used to detect the stress effects in green plants [33].

Chlorophyll fluorescence emission spectra of leaf measured at room temperature usually exhibits two emission maxima, the first maximum near 690 nm and the second one between 730 and 740 nm [15, 25,31]. In fully green leaves the two fluorescence maxima near 690 and 730 nm are of about equal intensity. In light green leaves the 690 nm fluorescence is much higher while the fluorescence maximum at 730 nm is present as a shoulder. With increasing Chl contents of a leaf, the relative fluorescence at 690 nm becomes smaller and the shoulder at 730 nm develops to a second maxima. This is due to the re-absorption of the emitted shorter wavelength fluorescence by the *in vivo* Chl, since the Chl absorption and fluorescence emission bands overlap in this region. As a consequence, the ratio F690/F730 exhibits lower value for fully green leaves than that observed in young developing or senescent leaves which have lower chlorophyll contents [20]. The chlorophyll fluorescence ratio F690/F730 is highly influenced by stress condition, due to decrease in chlorophyll contents and decline of photosynthetic activity [24, 25,30].

The dark-adapted (15–20 min) plant leaves upon illumination show induction kinetics which is known as Kautsky effect. Upon illumination, fluorescence increases spontaneously from ground fluorescence level ($F_0$) to fluorescence maxima ($F_m$) within 500 ms. With the onset of membrane energization and photosynthetic oxygen evolution, fluorescence decreases slowly and continuously (after 4–5 min) to reach a steady state level ($F_s$). In a darkened leaf, the photosynthetic apparatus is in the non-functional state-I, where the two photosynthetic photosystems are impaired. After light triggered fluorescence induction, the photosynthetic apparatus is in the photosynthetically active state-II, with a low yield of Chl fluorescence. Larger the slow fluorescence decrease ($F_d$) from $F_m$ to $F_s$, greater is the photosynthetic capacity of the leaf. The decrease in fluorescence from $F_m$ to $F_s$ is paralleled with increase in rate of oxygen evolution and photosynthetic $CO_2$ fixation [21]. The relative extent of fluorescence decrease is therefore an approximate measure of the degree of photosynthetic quantum conversion of a leaf. Several other factors may participate in this fluorescence decrease, e.g., thermal quenching, phosphorylation of the light-harvesting Chl protein, etc. [17,36]. The ratio of fluorescence decrease to the steady state fluorescence ($R_{fd} = F_d/F_s$), as calculated from the slow fluorescence decline kinetics at 690 and 730 nm are very suitable indicators of vitality and stress conditions of the plants, and have been termed as vitality index [24,43].

Incident light is absorbed by leaf, reflected from the leaf surface or transmitted unabsorbed through the leaf. When the content of photosynthetic pigments in leaf is low, the absorption of incident light is low and the reflectance and transmittance are high. Leaf reflectance provides a vast data resource for assessing plant health based on leaf biochemistry and anatomy due to the impact of biotic and abiotic stresses which in turn produce distinct changes in leaf optical properties. Reflectance characteristic of leaf in the region of 400–700 nm is primarily influenced by the cellular level of colored pigments like chlorophyll, anthocyanins and carotenoids [2,44]. There are two zones in the reflectance spectrum – a broadband around 550–600 nm and a narrow band around 700 nm, in the visible region. These two bands are most sensitive to changes in leaf Chl contents. There are many reflectance indices which are very sensitive and change linearly with leaf Chl contents of dark-green to yellow colored leaves [32]. One of these indices is Narrow Band Vegetation Index (NBVI; R750/R700) which shows a strong correlation with chlorophyll contents [27,32].

Cadmium, a non-essential heavy metal, is highly toxic to plants. It is mainly released in the environment during various agricultural, mining and industrial activities and also from automobile exhausts.

Cadmium inhibits photosynthesis. These inhibitory effects occur at multiple sites *viz.* Chl biosynthesis and degradation [8,42], PSI and PSII [6,7,39], disorganization of oxygen evolution complex and LHCII antenna system [5,18,28,38]. Cadmium directly affects the composition and structure-function relationship of thylakoids [7,28]. It also inhibits enzymes of the $CO_2$ assimilation pathway [41,45] and Rubisco activity [40]. Cadmium also decreases stomatal density and conductance [4].

The present paper deals with the study of effect of cadmium on overall growth, photosynthetic pigment contents and photosynthetic activity of wheat plants by using laser-induced chlorophyll fluorescence and reflectance spectroscopy.

## 2. Materials and methods

### *2.1. Plant growth and treatment with cadmium*

Healthy and uniform sized seeds of *Triticum aestivum* L. (Var. PBW 343) were surface sterilized in 4% sodium hypochlorite solution (v/v, in double distilled water) for 20 min and presoaked for 20 h in distilled water and kept wrapped in wet cloth overnight. Uniformly germinated seeds were selected and transferred into small pots containing acid washed sterilized sand (≈260–270 g). The seedlings were grown in a growth chamber under the photosynthetic photon flux density of 100 µmol photon $m^{-2}s^{-1}$ and 23 ± 2°C temperature with a 14:10 h light:dark photoperiod. After 3 days of germination, plants were irrigated with 0.2% modified Rorrison medium. The basic components of Rorrison medium are as follows: 0.4 mM $Ca(NO_3)_2$, 0.2 mM $MgSO_4 \cdot 7H_2O$, 0.2 mM $KH_2PO_4$, 0.1 µM $CuSO_4 \cdot 5H_2O$, 0.2 µM $ZnSO_4 \cdot 7H_2O$, 9.2 µM $H_3BO_3$, 1.8 µM $MnCl_2 \cdot 4H_2O$, 0.2 µM $NaMoO_4 \cdot 2H_2O$ and 10 µM FeEDTA. Cadmium treatment (0.01, 0.10 and 1.00 mM) was given to the plants along with nutrient medium on alternate days and the first treatment was given after six days of germination. Plant leaves were used for analyzing the effect of Cd after 10 days of the first treatment.

### *2.2. Determination of pigments*

Leaves (20 mg) from control as well as Cd treated *Triticum aestivum* L. plants were extracted in 3 ml 80% acetone (*v/v*, in double distilled water) and the extract was used for quantifying the pigment contents. The amount of Chlorophyll $a$, $b$ and carotenoids were determined by recording the absorbance in the region of 380–700 nm by using UV/Vis spectrophotometer (Perkin Elmer lambda 35) according to the method of Lichtenthaler and Welburn [26].

### *2.3. Laser-induced chlorophyll fluorescence spectra by violet diode laser (405 nm)*

LICF spectra were recorded using computer control Acton 0.5 Meter triple grating monochromator, Hamamastu R928 PMT as a detector. The samples were excited by a violet diode laser (Oxxus CE, made in France, model PS-001, wave length, 405 nm, Power, 50 mW) light. The beam expander was aligned to obtain 2 $cm^2$ expanded laser light on leaves. The fluorescence was collected on the entrance slit of the monochromator.

LICF spectra were recorded in the region of 650–780 nm with 1800 grooves/mm grating blazed at 500 nm wavelength using survey mode of spectra sense software. These spectra were analyzed using GRAMS 32 software with curve fit Array basic program. Spectral correction was made from the response curve of PMT and grating of monochromator.

### 2.4. Fluorescence induction kinetics by 635 nm red diode laser

Same experimental setup was used for the fluorescence induction kinetics (FIK) with 635 nm red diode laser (power 10 mW). The FIK curves were recorded by exposing 20 min pre dark-adapted plant leaves by red diode laser. The fluorescence intensity was recorded as a function of time at two different wavelengths 685 and 730 nm for 5 min using Intensity vs. Time mode of spectra sense software. Various parameters such as fluorescence maximum ($F_m$), steady state level ($F_s$) and fluorescence decrease ($F_d$) were calculated from these curves. Vitality index ($R_{fd} = F_d/F_s$) was also calculated from these parameters for all the samples.

### 2.5. Curve-fitting

Interactive non-linear curve fitting was done using Levenberg–Marquardt algorithm method. After choosing the Gaussian spectral function, the individual component peaks were selected. Peak widths were adjusted so as to match approximately the line shapes of the spectrum. It provides a reasonable matching fit of the spectral data with good F-statistics, standard error for peak amplitude, peak center and bandwidth (full width at half intensity maximum).

### 2.6. Reflectance spectroscopy

Optical reflectance spectra were measured at room temperature by using UV/Vis spectrophotometer (Perkin Elmer lambda 35) with an integrating sphere (50 mm Labsphere RSA-PE-20 integrating sphere with Hamamatsu S1227-66Q, Si photodiode as detector). SRS-99-010 Labsphere diffuse reflectance standard with reflectance factor of 99% was used as a standard. The spectrum was recorded by scanning the measuring light beam and by measuring the reflected light. Data were collected in the wavelength range of 400–800 nm with spectral resolution of 2 nm.

## 3. Results and discussion

### 3.1. Growth and photosynthetic pigments

Plant growth parameters and photosynthetic pigment contents are shown in Tables 1 and 2. Plants treated with 0.01 mM Cd showed an increase in plant growth over the control plants. Increasing concentration of Cd in the nutrient solution produced significant growth inhibition in wheat plants. For 0.01 mM Cd, the shoot and root lengths were stimulated by 2.11 and 5.0%, respectively, over the control plants.

Table 1
Plant growth parameters (shoot length, root length, fresh weight and dry weight) of control as well as Cd-treated wheat plants

| Treatment of Cd | Control | 0.01 mM | 0.10 mM | 1.00 mM |
|---|---|---|---|---|
| Shoot length (cm) | $20.84 \pm 0.12$ | $21.28 \pm 0.16$ (2.11) | $20.42 \pm 0.14$ (−2.01) | $17.96 \pm 0.12$ (−13.81) |
| Root length (cm) | $10.00 \pm 0.14$ | $10.5 \pm 0.13$ (5.0) | $9.8 \pm 0.14$ (−2.0) | $9.48 \pm 0.12$ (−5.2) |
| Fresh eight (g) | $1.49 \pm 0.13$ | $1.56 \pm 0.11$ (4.69) | $1.42 \pm 0.15$ (−4.69) | $1.36 \pm 0.09$ (−8.72) |
| Dry weight (g) | $0.215 \pm 0.14$ | $0.225 \pm 0.12$ (4.65) | $0.191 \pm 0.15$ (−11.16) | $0.179 \pm 0.13$ (−16.74) |

*Notes*: $\pm$ values indicate standard deviation (mean $n = 3$). The value in parenthesis shows percent decrease/increase over control plants.

Table 2
Photosynthetic pigment contents and pigment ratios of control as well as Cd-treated wheat plant leaves

| Treatment of Cd | Control | 0.01 mM | 0.10 mM | 1.00 mM |
|---|---|---|---|---|
| Chl $a$ (μg/ml) | $7.93 \pm 0.16$ | $9.58 \pm 0.18$ (20.8) | $7.46 \pm 0.16$ (−5.92) | $5.82 \pm 0.19$ (−26.6) |
| Chl $b$ (μg/ml) | $2.39 \pm 0.12$ | $2.86 \pm 0.13$ (19.66) | $2.14 \pm 0.15$ (−10.46) | $1.87 \pm 0.18$ (−21.75) |
| Total Chl (μg/ml) | $10.32 \pm 0.14$ | $12.4 \pm 0.16$ (20.63) | $9.61 \pm 0.16$ (−6.87) | $7.69 \pm 0.18$ (−25.48) |
| Car (μg/ml) | $1.45 \pm 0.16$ | $1.70 \pm 0.15$ (17.24) | $1.51 \pm 0.12$ (4.13) | $1.08 \pm 0.13$ (−25.21) |
| Chl $a/b$ | $3.31 \pm 0.15$ | $3.34 \pm 0.16$ (0.91) | $3.47 \pm 0.16$ (4.83) | $3.11 \pm 0.18$ (−6.04) |
| Chl/Car | $7.07 \pm 0.12$ | $7.30 \pm 0.13$ (3.25) | $6.32 \pm 0.15$ (−10.6) | $7.10 \pm 0.16$ (0.42) |

The toxic effect of Cd is more pronounced on shoot length than on root length. It is nearly 2.66 times higher for 1.00 mM Cd. The decrease in shoot length was 2.01 and 13.81% while, that in root length was 2.0 and 5.2% for 0.10 and 1.0 mM Cd over the control plants, respectively. Increase/decrease in fresh and dry weight of wheat plants was parallel to the increase/decrease in shoot and root length of plants. The increase in fresh and dry weight for 0.01 mM Cd was nearly equal (4.69 and 4.65%, respectively). However, for higher concentration of Cd there is greater decline in dry weight. The decline in dry weight for 0.10 and 1.00 mM Cd was 11.16 and 16.74% as compared to 4.09 and 8.72% decline in fresh weight, respectively. The inhibitory effect of Cd on plant elongation is mediated through altered cell growth. Cadmium in cells gets associated with cell wall and middle lamella and increases the cross linking between the cell wall components, resulting in inhibition of cell wall expansion and growth [37]. Moreover, Cd also alters the water relation in plants causing a physiological drought condition [3]. It is also responsible for metabolic dysfunction such as production of reactive oxygen species [1], and inhibition of photosynthesis [11,19] as well as nutrient uptake [34]. These and other such altered processes lead to decrease in shoot length, root length, fresh mass and dry mass of the plants subjected to Cd stress.

Similar to the growth response, the photosynthetic pigment contents also showed an increasing trend for 0.01 mM Cd while a reverse trend was observed for 0.10 and 1.00 mM Cd. The percentage increase in amount of Chl $a$ (20.8%) was slightly higher than that recorded for Chl $b$ (19.66%) in case of 0.01 mM Cd treated plants. However, the response of Cd to photosynthetic pigment contents at its toxic concentrations was varied. The decline in Chl $a$ content was less than that of Chl $b$ at 0.10 mM Cd but the response was reversed at 1.00 mM Cd (Table 2). The carotenoid (Car) contents showed an increasing trend for 0.01 and 0.10 mM, but it decreased by 25.21% for 1.00 mM Cd treatment. The Chl $a/b$ and Chl/Car ratios showed variable responses. Bazzaz and Govindjee [6] reported that Cd produced degradation of Chl and Car, inhibited their biosynthesis, and induced oxidative stress by disturbing the chloroplast. Thus, stimulated plant growth and increase in photosynthetic pigment contents indicate that 0.01 mM Cd induced growth in wheat plants. In contrast, inhibition of plant growth and pigment contents at higher concentrations (0.10 and 1.00 mM) confirmed the toxic effects of Cd at these concentrations.

### *3.2. Laser-induced chlorophyll fluorescence*

The curve fitted LICF spectra of control as well as Cd treated wheat leaves are shown in Fig. 1. Data related to Chl fluorescence parameters such as peak height, band width (full width at half maxima, FWHM) and band area of the curve fitted fluorescence spectra of treated and untreated wheat plants are given in Table 3 and graphically shown in Fig. 2. The LICF spectra of control as well as Cd treated wheat plants exhibited two fluorescence maxima, one in the red region near 685 nm and second in the far-red region near 730 nm. The fluorescence intensity at 685 and 730 nm of wheat plants is affected by Cd treatment. The effect of Cd treatment is more at 685 nm than at 730 nm, as indicated by fluorescence

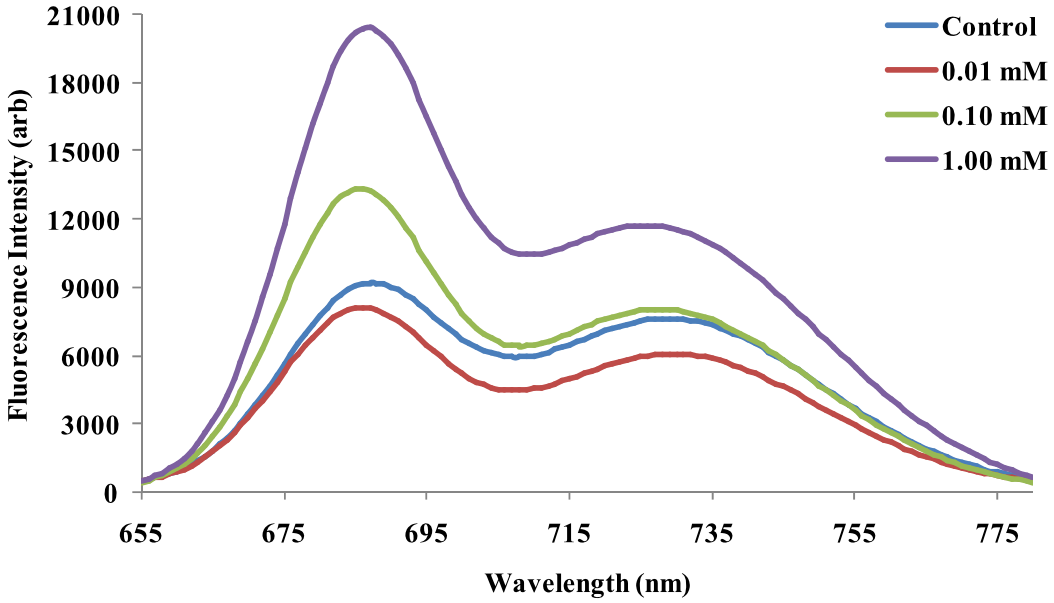


Fig. 1. Gaussian curve-fitted LICF spectra of control and Cd-treated wheat plant leaves excited by 405 nm violet diode laser. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0530.)

Table 3

Chlorophyll fluorescence parameter of the curve fitted spectra of control as well as Cd-treated wheat plant leaves excited by 405 nm violet diode laser

| Treatment of Cd | Control | 0.01 mM | 0.10 mM | 1.00 mM |
|---|---|---|---|---|
| Peak height | 0.72 ± 0.01 | 0.60 ± 0.01 (−16.5) | 0.87 ± 0.02 (20.39) | 0.95 ± 0.01 (31.06) |
| Band width[a] | 0.53 ± 0.01 | 0.54 ± 0.02 (1.89) | 0.48 ± 0.01 (−9.43) | 0.47 ± 0.02 (−11.32) |
| Band area | 0.54 ± 0.01 | 0.47 ± 0.02 (−12.97) | 0.60 ± 0.01 (11.11) | 0.63 ± 0.01 (16.67) |

*Note*: [a]Full width at half intensity maxima (FWHM).

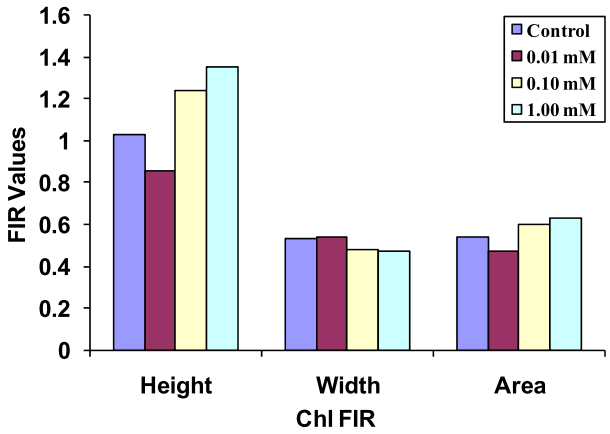


Fig. 2. Fluorescence intensity ratios (FIR) of the curve-fitted parameters of LICF spectra of control and Cd-treated wheat plant leaves exited by 405 nm violet diode laser. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0530.)

spectra. As a result, the FIR ratios of Cd treated wheat leaves showed much variation over the control plants. The increase of Chl fluorescence with increasing Chl concentration is mainly detected in the long wavelength range (far-red fluorescence). Short-wavelength red fluorescence first levels off and then decreases due to the re-absorption of the emitted red Chl fluorescence by the Chl absorption bands, which reduces the short-wavelength fluorescence with increasing Chl contents [9]. The effect of the decrease in Chl contents is mainly detected in short-wavelength range (red Chl fluorescence). This is because short-wavelength red Chl fluorescence increases with decrease in Chl contents due to reduction of re-absorption of the emitted red Chl fluorescence by the Chl absorption band. In green leaves about 90% of the emitted Chl fluorescence at 685 nm is reabsorbed by the Chl molecules of the leaf. This re-absorption is caused by the overlapping of short-wavelength range of the Chl fluorescence emission spectrum with the long-wavelength of Chl absorption spectrum [12]. Since the red Chl fluorescence maxima near 690 nm is more strongly affected by the re-absorption than the long-wavelength maximum near 730–740 nm, the ratio F685/F730 increases with decreasing Chl contents and *vice-versa*. Thus FIR is strongly influenced by variation in Chl contents and photosynthetic activity of the leaf [29,35]. The recorded FIR value decreases in case of 0.01 mM Cd treated plants suggesting that application of low concentration of Cd increases the Chl contents of the leaves. However, increase in FIR values for 0.10 and 1.00 mM Cd treatment shows that Chl concentration decreases at these concentrations.

### *3.3. Fluorescence induction kinetics*

FIK curves are shown in Fig. 3. The fluorescence decrease ratio or plant vitality indices are given in Table 4 and shown in Fig. 4. The $R_{fd}$ value shows an increase for 0.01 mM Cd. It increases by 4.32 and

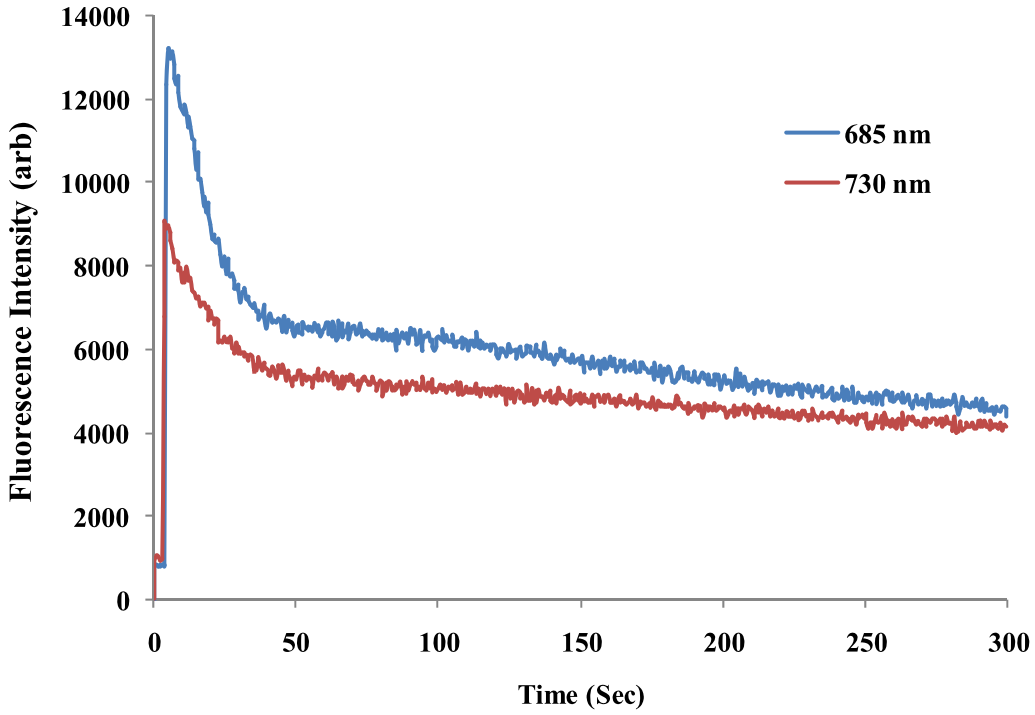


Fig. 3. Fluorescence induction kinetics curve of wheat plant leaves at 685 and 730 nm exited by 635 nm red diode laser. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0530.)

Table 4
Fluorescence-induction kinetics parameters for the control and Cd-treated wheat plant leaves at 685 and 730 nm excited by 635 nm red diode laser

| Treatment of Cd | Control | 0.01 mM | 0.10 mM | 1.00 mM |
|---|---|---|---|---|
| $R_{fd}$ 685 | 1.62 ± 0.01 | 1.69 ± 0.02 (4.32) | 1.56 ± 0.02 (−3.7) | 1.07 ± 0.01 (−33.95) |
| $R_{fd}$ 730 | 1.11 ± 0.02 | 1.22 ± 0.01 (9.9) | 1.00 ± 0.03 (−9.9) | 0.55 ± 0.02 (−50.45) |

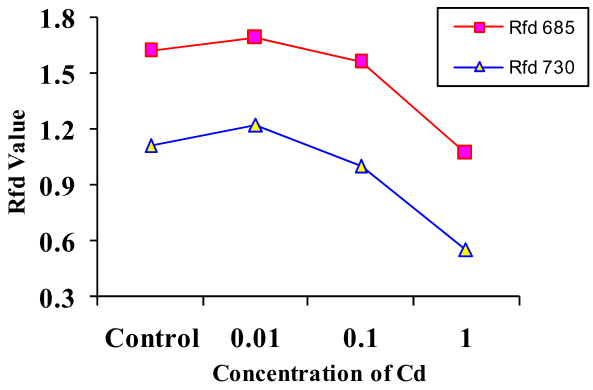


Fig. 4. Plant vitality index ($R_{fd}$) at 685 and 730 nm for control as well as Cd treated wheat plant leaves excited by 635 nm red diode laser. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0530.)

9.9% at 685 and 730 nm for 0.01 mM Cd over the control plants. The decrease in $R_{fd}$ values at 685 nm are 3.7 and 33.95% for 0.10 and 1.0 mM Cd, respectively, over the control plants. The decrease in $R_{fd}$ values at 730 nm are 9.9 and 50.25% for 0.10 and 1.00 mM Cd, respectively. With the onset of illumination in a 20 min dark-adapted leaf, the Chl fluorescence rises to maximum '$F_m$' within 500 ms and then decreases slowly to steady-state '$F_s$' level in 4–5 min. In the dark-adapted leaf, the primary accepters $Q_A$ and $Q_B$ are thought to be fully oxidized and the reaction centre of PSII is "open". Upon illumination, $Q_A$ becomes reduced and transfers the electron to $Q_B$ and during the increase to the maximum fluorescence ($F_m$) the plastoquinone pool is successively reduced by $Q_B$. At the point of the $F_m$, $Q_A$, $Q_B$ and PQ pool are fully reduced by PSII. The reaction centre of PSII is now "closed". With the onset of PSI activity (which reoxidizes the PQ pool as well as $Q_A$ and $Q_B$), $F_m$ slowly decreases to a terminal steady state [22, 23,25]. Thus, the value of $F_m$ is directly proportional to the plastoquinone pool size and the fluorescence decrease from $F_m$ to $F_s$ is paralleled by increase in rate of oxygen evolution and photosynthetic $CO_2$-fixation. The $R_{fd}$ value is a potential indicator of plants photosynthetic activity. The $R_{fd}$ value increases at 0.01 mM Cd. This suggests that application of low concentration of Cd increase the photosynthetic capacity and activity of wheat leaves. Moreover, at higher concentration of Cd (0.10 and 1.00 mM) the photosynthetic activity decreases subsequently.

### *3.4. Reflectance spectra*

The reflectance spectra of control as well as Cd treated wheat plants in the region of 400–800 nm are shown in Fig. 5. The narrow band vegetation indices (NBVI) are given in Table 5. NBVI shows an increase for 0.01 mM Cd and decrease for 0.10 and 1.00 mM Cd treated wheat leaves. The reflectance spectra of control as well as Cd treated wheat leaves show a broad band in the region of 500–600 nm and a narrow band near 678 nm. Reflectance characteristics of the leaf in the region of 400–700 nm

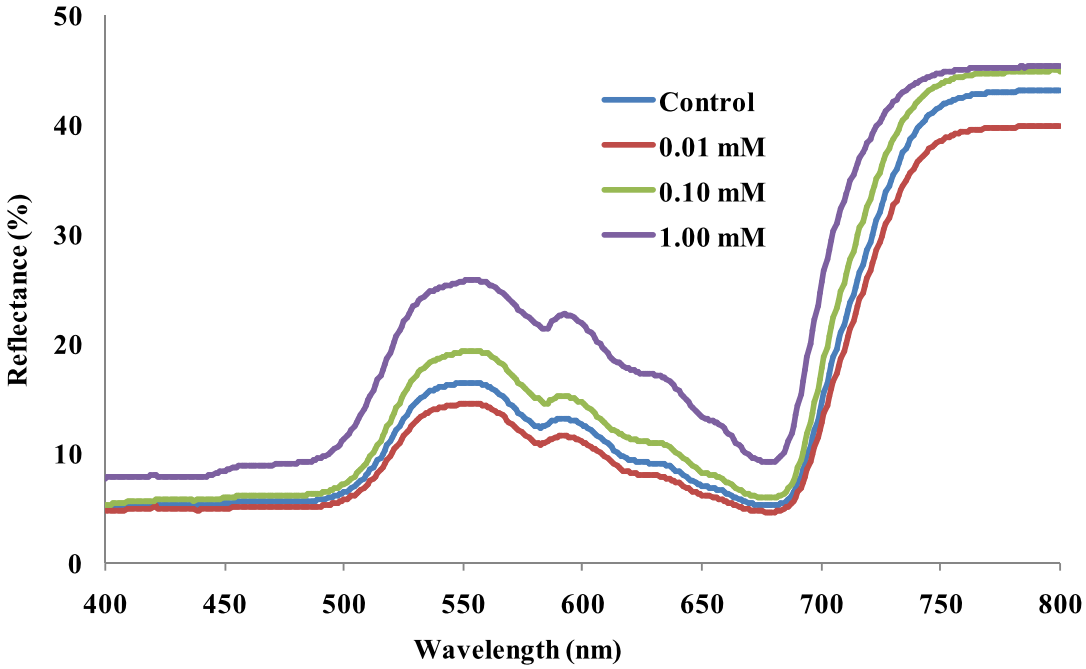


Fig. 5. Reflectance spectra of control and Cd-treated wheat plant leaves. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0530.)

Table 5
Narrow band vegetation index (NBVI; R750/R700) for the control and Cd-treated wheat plant leaves

| Treatment of Cd | Control | 0.01 mM | 0.10 mM | 1.00 mM |
|---|---|---|---|---|
| NBVI (R750/700) | 2.82 ± 0.04 | 3.01 ± 0.05 (6.74) | 2.47 ± 0.06 (−12.41) | 1.75 ± 0.04 (−37.94) |

are primarily influenced by the cellular level of colored pigments like chlorophyll, anthocyanins and carotenoids [2,44]. The reflectance spectra show considerable variation with Cd treatment over the control plants. The broad band in 500–600 nm range of reflectance spectra shows decrease and increase in height, with decrease and increase in Cd concentrations, respectively, as shown in Fig. 5. The percentage reflectance decreases for 0.01 mM and increases for 0.10 and 1.00 mM Cd as shown in the reflectance spectra. NBVI is directly correlated with Chl concentration [13,14], and it is considered as a very good stress indicator [27]. Sensitivity of NBVI to the chlorophyll contents of Cd treated wheat leaves are shown in Fig. 6. NBVI value increases for 0.01 mM Cd. This suggests that Cd at this concentration increases the Chl contents of the leaves. However, decrease in NBVI for 0.10 and 1.00 mM Cd indicates that Chl contents of wheat leaves decreases at higher concentrations of Cd.

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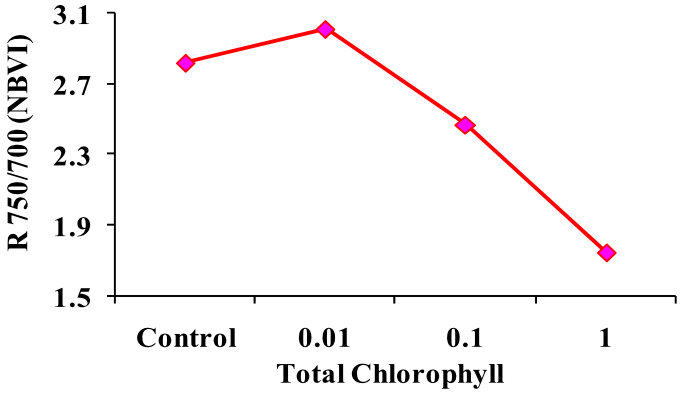


Fig. 6. Sensitivity of Narrow-band Vegetation Index (NBVI) to the chlorophyll contents for control as well as Cd-treated wheat plant leaves. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0530.)

## References

[1] K. Asada, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50** (1999), 601–639.
[2] J.L. Baltzer and S.C. Thomas, *Am. J. Bot.* **92** (2005), 214–230.
[3] J. Barcelo and C. Poschenrieder, *J. Plant. Nut.* **13** (1990), 1–37.
[4] A. Baryla, P. Carrier, F. Franck, C. Coulomb, C. Sahut and M. Havaux, *Planta* **212** (2001), 696–709.
[5] T. Baszynski, L. Wajda, M. Krol, D. Wolinka, Z. Krupa and A. Tukendorf, *Physiol. Plant* **48** (1980), 365–370.
[6] M.B. Bazzaz and Govindjee, *Environ. Lett.* **6** (1974), 1–12.
[7] J.M. Becerril, A. Munoz-Rueda, P. Aparicio-Tejo and C. Gonzales-Murua, *Plant Physiol. Biochem.* **26** (1988), 357–363.
[8] B. Boddi, A.R. Oravecz and E. Lehoczki, *Photosynthetica* **31** (1995), 411–420.
[9] C. Buschmann, *Photosynth. Res.* **92** (2007), 261–271.
[10] C. Buschmann, H. Prehn and H.K. Lichtenthaler, *Photosynth. Res.* **5** (1984), 29–46.
[11] L.K. Chaugh and S.K. Sawhney, *Plant Physiol. Biochem.* **37** (1999), 297–303.
[12] A.A. Gitelson, C. Buschmann and H.K. Lichtenthaler, *J. Plant Physiol.* **152** (1998), 283–296.
[13] A.A. Gitelson and M.N. Merzlyak, *J. Plant Physiol.* **148** (1996), 494–500.
[14] A.A. Gitelson, M.N. Merzlyak and H.K. Lichtenthaler, *J. Plant Physiol.* **148** (1996), 501–508.
[15] R. Gopal, K.B. Mishra, M. Zeeshan, S.M. Prasad and M.M. Joshi, *Current Sci.* **83** (2002), 880–884.
[16] H. Kautsky and U. Franck, *Biochem. Zeit.* **315** (1943), 176–206.
[17] G.H. Krause and E. Weis, *Photosynth. Res.* **5** (1984), 139–157.
[18] Z. Krupa, *Physiol. Plant.* **73** (1988), 518–524.
[19] Z. Krupa, G. Quist and N.P.A. Hurner, *Physiol. Plant.* **88** (1993), 626–630.
[20] H.K. Lichtenthaler, *J. Plant Physiol.* **131** (1987), 101–110.
[21] H.K. Lichtenthaler, in: *Application of Chlorophyll Fluorescence*, H.K. Lichtenthaler, ed., Kluwer Academic Publisher, Dordrecht, 1988, pp. 129–142.
[22] H.K. Lichtenthaler, in: *Application of Remote Sensing in Agriculture*, M. Steven and J.A. Clark, eds, Butterworths Scientific, London, 1990, pp. 287–305.
[23] H.K. Lichtenthaler, C. Buschmann and M. Knapp, *Photosynthtica* **43**(3) (2005), 379–393.
[24] H.K. Lichtenthaler, C. Buschmann, U. Rinderle and G. Schmuck, *Radiat. Environ. Biophys.* **25** (1986), 297–308.
[25] H.K. Lichtenthaler and U. Rinderle, *CRC Crit. Rev. Anal. Chem.* **19** (1988), S29–S85.
[26] H.K. Lichtenthaler and A.R. Welburn, *Biochem. Soc. Trans.* **11** (1983), 591–592.
[27] H.K. Lichtenthaler, O. Wenzel, C. Buschmann and A. Gitelson, *Ann. N. Y. Acad. Sci.* **851** (1998), 271–283.
[28] W. Maksymiec and T. Baszynski, *Acta Soc. Bot. Pol.* **57** (1988), 465–474.
[29] R. Maurya and R. Gopal, *Appl. Spectrosc.* **62**(4) (2008), 433–438.
[30] R. Maurya, S.M. Prasad and R. Gopal, *J. Photochem. Photobiol. C Photochem. Rev.* **9** (2008), 29–35.
[31] K. Maxwell and G.N. Johnston, *J. Exp. Bot.* **51** (2000), 659–668.
[32] M.N. Merzlyak, A.A. Gitelson, S.I. Pogosyan, O.B. Chivkunova, L. Lehimena, M. Garson, N.P. Buzulukova, V.V. Shevyreva and V.B. Rumyantseva, *Russian J. Plant Physiol.* **44**(5) (1997), 614–622.
[33] K.B. Mishra and R. Gopal, *Int. J. Rem. Sen.* **29**(1) (2008), 157–173.
[34] H. Obata, N. Inoue and M. Umebayshi, *Soil Sci. Plant Nut.* **42** (1996), 361–366.

[35] J.K. Pandey and R. Gopal, *J. Fluoresc.* **21** (2011) 785–791.
[36] G. Papageorgiou, in: *Bioenergetics of Photosynthesis*, Govindjee, ed., Academic Press, New York, NY, USA, 1975, pp. 319–372.
[37] C. Poschenrieder, G. Gunse and J. Barcelo, *Plant Physiol.* **90** (1989), 1365–1371.
[38] F. Roynet and R. Lannoye, in: *Advances in Photosynthetic Research*, Vol. 4, C. Sybesma, ed., Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, 1984, pp. 443–446.
[39] A. Siedlecka and T. Baszynski, *Physiol. Plant* **87** (1993), 199–202.
[40] A. Siedlecka, Z. Krupa, G. Samuelsson, G. Oquist and P. Gardestrom, *Plant Physiol. Biochem.* **35** (1997), 951–957.
[41] M. Stiborova and S. Leblova, *Photosynthetica* **19** (1985), 500–503.
[42] A.K. Stobart, W.T. Griffiths, I. Ameen-Bukhari and R.P. Sherwood, *Physiol. Plant* **63** (1985), 293–298.
[43] R.J. Strasser, in: *Proceedings of International Geoscience and Remote Sensing Symposium*, Zurich, Vol. III, ESA Publications Division, Noordwijk, 1986.
[44] T.C. Vogelman, *Annu. Rev. Plant Phys.* **44** (1993), 231–251.
[45] H.J. Weigel, *Physiol. Plant* **63** (1985), 192–200.