Concentration dependences of the red to far-red fluorescence ratio of chlorophyll in higher plants

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We study the dependence of F_{682}/F_{734} parameter, the red to far-red fluorescence intensity ratio, on chlorophyll concentration under conditions of continuous illumination of vegetation in the process of growth and natural photoperiod. It is shown that the dependence of the F_{682}/F_{734} ratio on the chlorophyll content is described by a quadratic expression for vegetation grown under continuous illumination condition, and by a power-law function for naturally grown plants. The third type of concentration dependence of the F_{682}/F_{734} ratio is obtained for poplar leaves during the growth period. This dependence is more complicated, and cannot be described by a power-law or a quadratic function. It is concluded that all the dependences found thus far apply under specific conditions of growth, and that there is no universal function relating the red to far-red intensity ratio of the fluorescence to pigment abundance in higher plant leaves. The results presented here are well explained by the model, in which each photosystem emits at a specific wavelength.

Introduction

The fluorescence of chlorophyll α of a green leaf has two maxima in the red spectral range.[1–3] There are two viewpoints with regard to the mechanism of fluorescence onset in the wavelength ranges 680–685 nm (F_{682}) and 730–740 nm (F_{734}). The first one assumes that the double-peaked fluorescence spectrum is associated with the phenomenon of radiation reabsorption followed by Stokes shift of the reemitted radiation. In this model, the photosystem II (PS II), having the fluorescence band at 680–685 nm, is considered to be the fluorescence source. Radiation with $\lambda = 680–685$ nm is then absorbed by the photosystem I (reabsorption), and thus absorbed energy is reemitted at 730 nm.[2,4] The second viewpoint suggests that the fluorescence at 685 nm is caused by chlorophyll PS II, while that at 730 nm by chlorophyll PS I.[3,5,6]

It has been found[2–5] that the red to far-red intensity ratio depends on pigment abundance. At the same time, all the studies of variations of concentration dependences of the F_{682}/F_{734} ratio were performed using simultaneously taken samples of leaves grown under conditions of natural photoperiod. The obtained behavior of F_{682}/F_{734} ratio plotted versus pigment concentration was associated with reabsorption of emission from chlorophyll α.[2,4] Also, it was assumed that the chlorophyll PS I emits insignificant fraction of fluorescence at 734 nm, and so it can be neglected.[2,4] However, the literature data compiled up to now contradict the view of reabsorption-type emission of chlorophyll in leaves of plants[7,8] and the view of the contribution of PS I fluorescence to the total fluorescence signal.[9] Most recent data suggest that PS I may contribute up to 35% to the total fluorescence at 734 nm.[9] In addition, it is shown that the red to far-red fluorescence ratio depends on the intensity and length of illumination period during the plant vegetation period.[10,11] Summarizing, this paper is aimed at studying the dependence of the F_{682}/F_{734} ratio on the chlorophyll abundance under different illumination conditions of plant to obtain arguments in favor of one or another model of the fluorescence peaks occurrence.

Methods used in the study

We concentrated on leaves of cucumbers (*Cucumis sativum L.*) of Moskovskii Teplichny brand and leaves of a balsamic poplar (*Populus babsamifera*). The cucumber plants were grown in two ways: planted out in open soil near Krasnoyarsk (56°00′ N, 92°45′ E) in the period from July to late August and in vegetation boxes under controllable conditions using hydroponics on claydite under continuous illumination (with the intensity of 120 W/m^2). As a source of light, we used a DKsTV-6000 lamp, whose emission spectrum is close to the solar one. The poplar leaves were sampled in May–October 2001 on the territory of Krasnoyarsk academic town. The formed leaves were selected from 10 trees at 12:00 LT once every 3–4 weeks. The dependence of F_{682}/F_{734} ratio on the total chlorophyll concentration was studied under three illumination conditions: (1) during continuous illumination in the process of growth; (2) for a fixed light and dark regime (in this case, sampling for analysis of fluorescence parameters have been performed simultaneously from leaves of different levels having different colors); and in a variable light/dark regime, when a change of photoperiod is determined by the solar cycles.

The fluorescence intensity parameters of chlorophyll of plant leaves were measured using a fluorimeter,

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whose description and measurement technique are given in Ref. 12. Intensity of the exciting radiation was 180 W/m^2.

Before recording the fluorescence, the leaf cuts were kept in darkness in a Petri dish with wet filter paper during 10–15 min.[2] The luminescence was recorded at room temperature.

The state of photosynthetic mechanism of plant leaves was estimated using the stress adaptation index, determined from the formula presented in Ref. 13:

$$A_p = 1 - \frac{1 + R_f(734)}{1 + R_f(682)},\ R_f = (F_m - F_s)/F_s,$$

where F_m and F_s are maximum and stationary levels of the fluorescence[2]; given in parentheses is the wavelength of fluorescence. The photosynthetic pigments were extracted from the leaves using 96% ethanol, while pigment concentrations were determined using extinction coefficients measured.[14] The pigment content per unit leaf area was in mg/dm^2; and for the purposes of graphical presentation, the dependence of the F_{682}/F_{734} ratio on the sum concentration of α and β chlorophylls was normalized to 1 mg/dm^2. The fluorescence parameters measured and the pigment concentration were statistically processed.

Results

The dependence of the F_{682}/F_{734} ratio on the sum concentration α and β chlorophylls, grown under continuous illumination, is shown in Fig. 1. As seen, it is well fitted by quadratic equation

$$F_{682}/F_{734} = 0.064x^2 - 0.708x + 2.252;\ R^2 = 0.96, \quad (1)$$

where x are numeric values of the total concentration of α and β chlorophylls (in mg/dm^2), and R^2 is the correlation coefficient.

Under these conditions, the distribution of total chlorophyll content in leaves at different levels of vegetation was characterized as follows. The concentrations ranged from 4.5 to 6 mg/dm^2 for the first and second levels, from 6 to 7 mg/dm^2 for the third and fourth levels, and from 3.5 to 4.5 mg/dm^2 for the fifth and sixth levels.

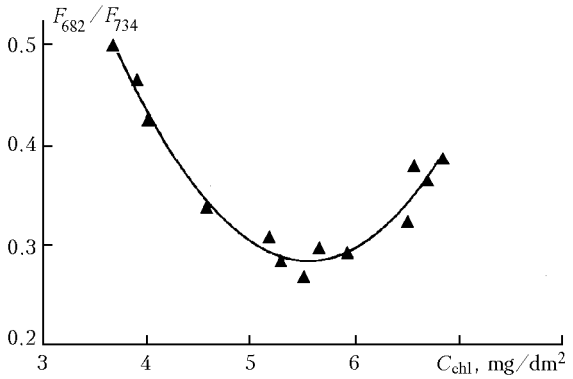

Fig. 1. The F_{682}/F_{734} ratio versus concentration of the sum of chlorophylls C_{chl} for cucumber plants grown under continuous illumination.

For plants grown in open soil under natural conditions, i.e., in a fixed light/dark regime, there is an inversely proportional dependence of the F_{682}/F_{734} ratio on the pigment content (Fig. 2). This curve is well fitted by the following formula:

$$F_{682}/F_{734} = 0.91x^{-0.46};\ R^2 = 0.92. \quad (2)$$

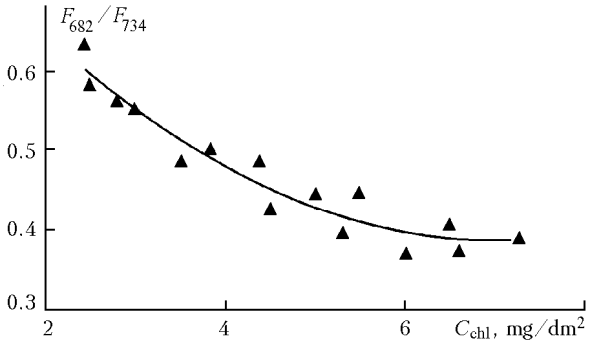

Fig. 2. Dependence of the F_{682}/F_{734} ratio on the concentration of the sum of chlorophylls C_{chl} when cucumber plants are grown in open soil under natural illumination.

From the curves in Figs. 1 and 2 it is seen that, for a given chlorophyll concentration in the range from 4 to 7 mg/dm^2, the F_{682}/F_{734} ratio appears to be lower under continuous illumination. Study of variations of the fluorescence F_{682} and F_{734} under varying chlorophyll content in plant leaves under conditions of different photoperiods has shown that F_{682} can be described by the function

$$F_{682} = 62.69e^{-0.672x};\ R^2 = 0.90 \quad (3)$$

for a discontinuous illumination, and by the function

$$F_{682} = 120.43e^{-0.665x} + 7.12;\ R^2 = 0.89 \quad (4)$$

for a continuous illumination.

From Eqs. (3) and (4) it follows that the concentration-induced variations of the intensity of fluorescence at 682 nm practically does not depend on the illumination regime (discontinuous or continuous).

The variations of the fluorescence intensity at 734 nm with varying chlorophyll content is well fitted by the equation

$$F_{734} = 0.28x^2 - 3.86x + 44.30;\ R^2 = 0.92 \quad (5)$$

for a natural photoperiod, and by the equation

$$F_{734} = -1.33x^2 + 8.72x + 25.68;\ R^2 = 0.93 \quad (6)$$

for a discontinuous illumination. As seen, the branches of parabolas, given by functions (5) and (6), go in opposite directions.

Therefore, the behavior of curves, described by equations (1) and (2), is determined primarily by variations of the fluorescence intensity at 734 nm.

The concentration and time dependences of the F_{682}/F_{734} ratio during variable dark/light regime in the vegetation process were studied for poplar leaves.

Variations of the F_{682}/F_{734} ratio in the process of vegetation of leaves is shown in Fig. 3. The F_{682}/F_{734} ratio practically did not suffer rapid variations until September, and only in October it increased by a factor of two. This period was characterized by frosts at nighttime, and by a change of leaf color from green to yellow.

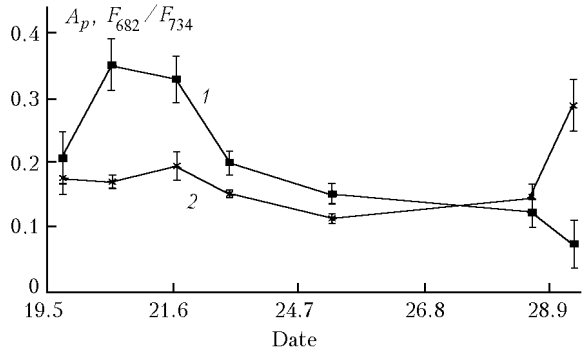

Fig. 3. Variations of stress adaptation index (*1*) and F_{682}/F_{734} ratio (*2*) in the process of vegetation of poplar leaves.

The state of the photosynthetic system of tree leaves was judged from stress adaptation index. As seen from Fig. 3 the index A_p is largest in June when the leaves are green and have no pathological changes. In the subsequent months, the index permanently decreased. At the same time, morphologic changes and parasitizing organisms *Phyllonoryeter populifoliella* and *Pemphigus bursarius* have appeared on the tree leaves. Before the trees go to rest, A_p reached its minimum value, and even was zero for some poplars, indicating toward irreversible changes occurred in the process of photosynthesis of tree leaves.[13] During tree vegetation, A_p and the F_{682}/F_{734} ratio showed different, uncorrelated variations.

The dependence of F_{682}/F_{734} ratio on the chlorophyll concentration in leaves in the process of poplar vegetation is shown in Fig. 4. It has quite a complicated character.

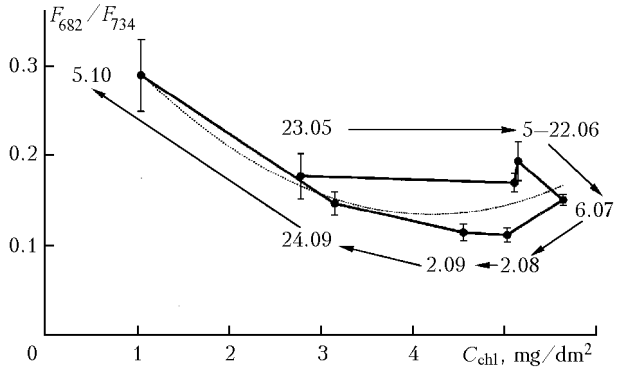

Fig. 4. Dependence of the F_{682}/F_{734} ratio on the chlorophyll concentration C_{chl} in poplar leaves in the process of vegetation (solid line) and its quadratic fit (dashed line). Digits are dates of sampling, and arrows indicate time sequence.

Approximation of data in Fig. 4 by functions found in Refs. 2, 3, and 5 gave the following correlation coefficients:

$$y = 0.27x^{-0.39};\ R^2 = 0.53;$$

$$y = 0.0156x^2 - 0.13x + 0.41;\ R^2 = 0.79.$$

Mathematically, the quadratic equation rather well fits the dependence of the F_{682}/F_{734} ratio on the chlorophyll content; however, the parabola falls within the confidence interval only at three data points from the total eight points. Therefore, the concentration dependence in Fig. 4 differs from the functions used above: power-law and quadratic.

Discussion

The results obtained for growing cucumber plants indicate that the illumination regime (continuous or discontinuous) influences the shape of concentration dependence of the fluorescence intensity at F_{734}, but not that of the fluorescence F_{682} [Eqs. (3)–(6)]. As a consequence, the variations of the F_{682}/F_{734} ratio as a function of chlorophyll concentration are described by a parabolic function in the case of high-intensity continuous illumination [Eq. (1)], and by a power-law function in the case of natural illumination [Eq. (2)].

For plants grown under conditions of continuous illumination, the F_{682}/F_{734} ratio was minimum in low-level leaves and increased in midlevel (mature) and high-level (young) leaves. For plants grown under natural illumination conditions, this parameter was minimum in mature midlevel leaves, in contrast to young and old leaves that fully agrees with the conclusions drawn for deciduous trees.[15] Thus, the F_{682}/F_{734} ratio is uniquely related neither to the age nor to the concentration of the chlorophylls (see Fig. 1). The phenomenon of reabsorption cannot account for the behavior of the curve. It is quite possible that the reabsorption effect does take place under certain conditions; however, when plants are grown under continuous illumination, they experience structural and functional changes, capable of more significantly influencing the shape of fluorescence spectrum of chlorophyll α in leaves than the reabsorption.

The function presented in Fig. 4 is set using two parameters: chlorophyll content and time of vegetation. Here, the dependence of the F_{682}/F_{734} ratio on the chlorophyll content has a somewhat different shape. First, this is because of a different method of sampling leaves for the measurements. In our studies the samples were collected in the process of vegetation of plants, while in Refs. 2 and 4 differently colored leaves were sampled simultaneously. Second, in the process of vegetation of poplar leaves the length of the day and, hence, the duration of solar illumination of plants changed (from 11 to almost 18 h) from one measurement series to another; whereas in earlier studies they remained unchanged.

As known, the duration and intensity of illumination influence the structural arrangement of chloroplasts and, as a consequence, the energy of longwave fluorescence.[10] For this reason, the measurements with nearly the same chlorophyll concentration (5.03–5.15 mg/dm^2) but different

length of the day gave the following fluorescence intensity ratios F_{682}/F_{734}: 0.17 ± 0.01 on June 5; 0.19 ± 0.02 on June 22; and 0.11 ± 0.01 on August 2 (see Fig. 3). As the pigment content in leaves (and hence reabsorption effect) remained practically the same, the lower F_{682}/F_{734} ratios in August were either due to physiological process or to structural changes in photosynthetic mechanism.

The known stress factor influencing the F_{682}/F_{734} value is the water deficit in plants. However, analysis of leaves, picked and dried in air for 36 h, has revealed no more than 25% change in the ratio of peaks of red fluorescence signals of chlorophylls.[1] In our case, in poplar leaves the intensity ratio F_{682}/F_{734} was found to be almost two times lower in August than in June.

Thus, the dependence of the F_{682}/F_{734} ratio on concentration of chlorophylls, varying in the process of vegetation, probably reflects the PS II/PS I ratio in photosynthetic mechanism of leaves at definite times of tree development. If assuming that fluorescence at 734 nm wavelength is an indicator of PS I while that at 682 nm of the PS II, an increase in the F_{682}/F_{734} ratio observed in October is likely associated with a considerable quantitative predominance of PS II over PS I in photosynthetic mechanism of the leaves before their fall. This agrees with data of Sõnoike demonstrating higher sensitivity of PS I to chilling than that of PS II.[7,8] In studying the photosynthetic mechanism of leaves of higher plants, grown under different illumination conditions, it is necessary to analyze both the F_{682}/F_{734} ratios and A_p, since the former characterizes variations occurring in the second and first photosystems, and the latter one characterizes the damages in photosynthetic mechanism as a whole. High A_p values signify irreversible damage to chloroplasts of the plants.

Conclusion

We have considered three types of concentration variations of the ratio of red to far-red fluorescence intensity. All the dependences of the ratio F_{682}/F_{734} on chlorophyll concentration, revealed thus far, apply for specific conditions of plant growth and the method of data sampling used in measurements; moreover, there is no universal function relating the red-to-far-red fluorescence ratio to pigment content in leaves of higher plants, as is argued in Refs. 2 and 4. The results presented in the paper are well explained by the model that assumes each photosystem to emit at a specific wavelength. One of the main arguments against this theory relies on the fact that an isolated photosystem I has no fluorescence at room temperature; however such an argumentation neglects the mergence effect. A photosynthetic system may acquire new properties, features, and states, characteristic of none of the constituents. In this regard, it is of interest to study the concentration dependence of red and far-red fluorescence using pulsed-laser excitation of the luminescence in plants.[16,17]

Acknowledgments

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