Correlation between concentration of the photosystem I reaction centers and the far-red/red fluorescence ratio for phototroph chlorophyll

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Literature data on the dependence of the far-red/red ratio of fluorescence intensities measured at the temperature of liquid nitrogen in the culture of cyanobacteria and pea leaves, as well as our experimental results on determining fluorescence signals obtained at the room temperature on wheat leaves, on the number of reaction centers of photosystem I, have been analyzed. With increase of the concentration of reaction centers, the ratio of maxima of the red chlorophyll fluorescence grows linearly. The conclusion is inferred that the obtained dependence is characteristic for all photosynthesizing organisms, evolving oxygen.

The fluorescence of leaf chlorophyll is characterized by the double-peak structure. One peak is inside interval 682–686 nm (F_{682}), the other is in interval 730–742 nm (F_{734}).[1–3] The fluorescence intensity for each maximum is measured first, and then their ratio is calculated. The ratios F_{734}/F_{682} depend on the measurement temperature and the exciting light wavelength.[2,4] Some researchers believe that this parameter correlates with the chlorophyll concentration,[2–5] while others think that it is a distinguishing feature of a species.[1] Variation of F_{734}/F_{682} at different physiological states of plants is reported in Ref. 4. As it follows from Refs. 2–5, there is no unambiguous interpretation of the connection between F_{734}/F_{682} and other parameters, characterizing the development of plants.

Some results, obtained recently, allowed one to advance in interpretation of the information content of F_{734}/F_{682} ratio. First, it has been demonstrated that the contribution of photosystem I (PhS I) into the total fluorescence exceeds 40% at the room temperature.[6,7] Second, some new data point to connection between the increase of the far-red/red fluorescence ratio and the increase of PhS I (P_{700}) concentration of reaction centers at a temperature of liquid nitrogen.[8] Third, experiments have shown that the fluorescence signal characterizes photosystem II (PhS II) at 685 nm and PhS I at 730 nm.[9] Since the contribution of PhS I into the total fluorescence at the room temperature has been considered insignificant up to recently, interrelations of fluorescence parameters with the number of components of photosystem I have not been investigated up to recently.

In this paper the connections between the far-red/red fluorescence ratio of phototroph chlorophyll and the concentration of reaction centers of photosystem I are considered.

Objects and methods of the study

The wheat 232 (*Triticum aestivum* L) was taken as the object of the study. The plants were grown in hermetic phytotrons under controlled conditions by the method of hydroponics on claydite under continuous illumination (photosynthetic active radiation of 150 W/m^2, air temperature of 24°C). The DKsTV-6000 lamps were used as a source of radiation.

To determine the concentration of PhS I reaction centers, the chemoinduced method[10] was used.

The intensity of leaf chlorophyll fluorescence at wavelengths of 682 and 734 nm was measured by a two-wave fluorimeter.[11]

Besides, to analyze the connection between F_{734}/F_{682} and P_{700}, the literature data were used.

Results and discussion

In 1987, it was reported[12] on studies of the fluorescence spectra of pea leaves and chloroplasts, as well as of the number of reaction centers of the both photosystems under deficit of iron. It has been established that the deficiency of iron in plant nutrition causes the chlorosis, which begins to manifest itself only in 3–4 weeks of plant growth. The content of pigments in the leaves of the 7th layer did not exceed 15–20% of the same changes in test plants. Fluorescence measurements were used in that work only to demonstrate the presence and activity of the reaction centers of photosystems I and II. The authors based on the fact that every chlorophyll-albumen complex had specific radiation bands of the low-temperature fluorescence.

In parallel, the number of the photosystem reaction centers was measured from the magnitude of

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light-induced signals of the electron paramagnetic resonance.

However, the correlation analysis between these parameters was not conducted. The concentration dependence was constructed by the results of Ref. 12 (Fig. 1).

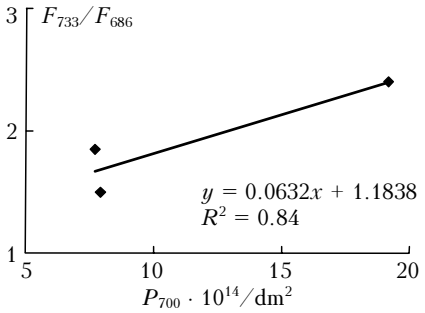

Fig. 1. The ratio F_{733}/F_{686} as a function of concentration of PhS I reaction centers in pea leaves.[12]

It is seen that the connection between F_{733}/F_{686} and the number of reaction centers P_{700} is approximated by a straight line.

Later, the influence of root hypoxia and the deficiency of iron on the spectral properties and the number of reaction centers of pea photosystems was studied,[13] and it has been demonstrated that such a limitation causes simultaneous changes in low-temperature fluorescence characteristics, the number of reaction centers, and the chlorophyll concentration. As in Ref. 12, the analysis of correlation between these parameters was not made. The study of the connection between F_{733}/F_{686} and P_{700} made it possible to conclude that the correlation is of the same character as in Ref. 12 (Fig. 2).

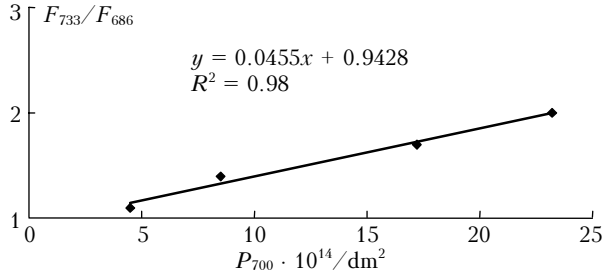

Fig. 2. The ratio F_{733}/F_{686} as a function of concentration of PhS I reaction centers in pea leaves.[13]

Due to new facts about the PhS I contribution into the fluorescence and the connection between the far-red fluorescence and PhS I, a cycle of experiments with the cyanobacteria was conducted. For instance, the dependence the far-red/red fluorescence ratio and the number of PhS I reaction centers on the time of *Synechococcus sp.* cultivation in an iron-deficient medium was considered in detail.[14] As in the previous papers, the dependence of the fluorescence parameter concentration on P_{700} content was not constructed. The study of the dependence shows that it is linear (Fig. 3).

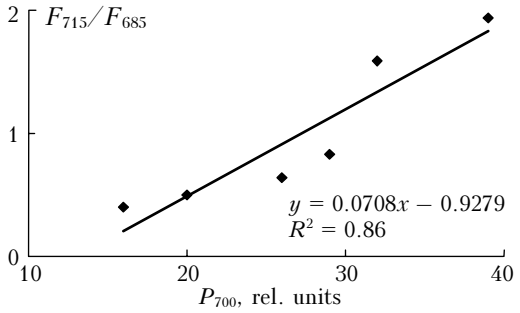

Fig. 3. The ratio F_{715}/F_{685} as a function of concentration of PhS I reaction centers in cells of cyanobacteria.[14]

As it follows from the analysis of literature data, the ratio of red fluorescence intensities at the temperature of liquid nitrogen is directly proportional to the number of reaction centers of PhS I. This dependence is similar both for algae and plants.

All the aforesaid is connected with measurements of the low-temperature fluorescence. According to Ref. 11, it is possible to obtain chlorophyll fluorescence spectra of phototrophs at the room temperature, which are similar to those obtained for the low-temperature fluorescence. To do this, it is necessary to excite fluorescence by a wide-band (380–540 nm) light with an intensity of 180 W/m^2. Dependences of fluorescence parameters on the concentration of PhS I reaction centers under given measurement conditions have not been measured earlier. Below we present such a study, performed with wheat leaves.

The dependence of F_{734}/F_{682} on concentration of PhS I reaction centers were studied, using 12–14-day wheat seedlings. All leaves, but from the first layer, were used in the measurement. Raw mass variations per unit of a leaf surface (excised) did not exceed 30%. For instance, raw mass of the excision varied in one of the experiments from (10.1 ± 0.7) to (13.0 ± 0.7) mg for the leaves of the 2nd–5th layers. As is seen in Fig. 4, the spread of the experimental points is relatively large. This can be explained by different masses of leaf blades. Such a 30%-variation in excised raw mass has not any effect on the linear connection between F_{734}/F_{682} and concentration of PhS I reaction centers in wheat leaves of different layers.

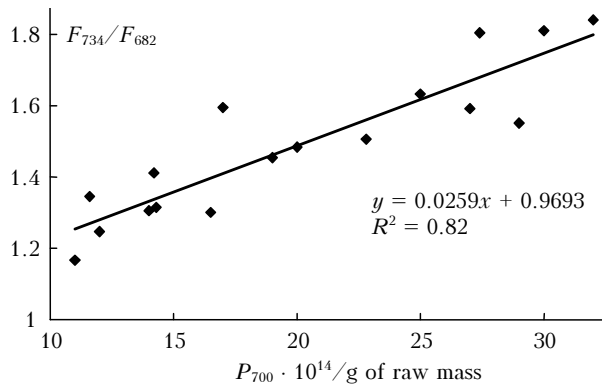

Fig. 4. The ratio F_{734}/F_{682} as a function of concentration of P_{700} reaction centers in wheat leaves of different layers.

With the increase of the concentration of P_{700} reaction centers, the ratio of maxima of chlorophyll red fluorescence increases linearly even at the room temperature. The obtained dependence is identical to that for pea (see Figs. 1, 3). The difference is that spectra for pea and wheat are obtained at low temperature and at the room one, respectively. To measure low-temperature leaf fluorescence spectra is a more difficult work, therefore, it can be accepted that the method of measuring chlorophyll fluorescence at a room temperature[11] is more convenient and takes less time to obtain fast information on the number of reaction centers in leaves. The comparison shows the method from Ref. 11 to be more than 30-fold faster relative to the chemoinduced method.[10]

Based on data from Refs. 12–14, where the number of photosystem I reaction centers is shown to be linearly connected with the far-red/red fluorescence ratio under low temperature for different kinds of photosynthesizing organisms, it can be supposed that measurements of fluorescence parameters at a room temperature can be extended to photosynthesizing objects, which release oxygen in the process of photosynthesis.

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