RESEARCH ARTICLE | FEBRUARY 20 2024

Influence of growth environment on structural and optical characteristics of higher plants

E. Bukhanov; V. N. Shikhov; V. V. Velichko; A. G. Lipshin; N. A. Surin

AIP Conf. Proc. 2924, 040003 (2024)
https://doi.org/10.1063/5.0181655

Influence of Growth Environment on Structural and Optical Characteristics of Higher Plants

E. Bukhanov,[1,2, a)] V. N. Shikhov,[3, b)] V. V. Velichko,[3, c)] A. G. Lipshin,[4, d)]
N. A. Surin[4, e)]

[1)] *Kirensky Institute of Physics FRC «KSC of SB RAS», 660036, Krasnoyarsk, Russian Federation*
[2)] *Federal Research Center «KSC of SB RAS», Krasnoyarsk, Russian Federation*
[3)] *Institute of Biophysics FRC «KSC of SB RAS», 660036, Krasnoyarsk, Russian Federation*
[4)] *Krasnoyarsk Research Institute of Agriculture FRC «KSC of SB RAS», 660041, Krasnoyarsk, Russian Federation*

[a)] *Corresponding author: k26tony@ya.ru*
[b)] *: v_shikhov@ibp.ru*
[c)] *l: vladimir_Velitchko@hotmail.com*
[d)] *: alipshin@mail.ru*
[e)] *: surin-akad@sh.krasn.ru*

Abstract. Two barley samples were taken for the study – one grown in the field and the other in a special chamber under intensive light. Chloroplasts structure was obtained during electron microscopy process. The results showed that the maximum quantum yield of photosystem II in both samples was in the range of values typical for normal physiological state of plants. At the same time, electron transport speed comparison shows that electrons are transferred 1.7 times faster in the vegetation chamber compared to field environment. Such difference is confirmed by the results of microscopy performed with flag leaves tissue samples of barley grown under intensive light in a chamber and in the field. A grana structure significantly denser and better organized was observed in chloroplasts of barley grown in chamber. This study shows a clear connection between the environment, the ordering of thylakoid structures and fluorescent indicators of photosynthesis.

INTRODUCTION

A distinctive characteristic of higher plants chloroplasts is cylindrical disk-shaped grana orderly organized in it. In such structures, strong interactions between exciton, Brag and lattice resonances may occur [1, 2, 3]. The results of recently published work [4] proved that higher plants indeed have an ordered structure inside, where the period is comparable to the visible range wavelength. It was also shown that density of photon states increased in efficient photosynthesis zone due to the fact that the local amplitude is larger than the amplitude of the incident wave (local amplification).

According to Fermi's golden rule, the rate of chemical reactions under the light is proportional to photon states density. We must also remember that the probability of a photochemical reaction depends on the local amplitude of electric field. The connection between photosynthesis and electric field was first proposed by Witt [5, 6].

Higher plants, unlike algae and other simple photosynthesizing bodies, have evolved a multilayer membrane structure that is highly sensitive to external influences. This is possible due to changes in the ratio of alternative electron transport pathways, ROS accumulation and induction of appropriate signaling pathways that affect gene expression. They also are able to dissipate energy better [7, 8, 9]. Barley stands out, with its high tillering and ability to place several ears on one stem (Fig. 1).

MATERIALS AND METHODS

In our study we examined barley plants of Emelya and Takmak varieties of the Krasnoyarsk Agricultural Institute breeding. The barley was grown in the field and under controlled conditions of intensive light culture. Under light culture conditions, plants were grown in sealed vegetation chambers on a neutral substrate with Knop's medium used as a nutrient solution. The photoperiod duration of artificial light varied during the growing season to simulate the

Proceedings of the 3rd International Conference on Nanomaterials, Nanofabrication and Nanocharacterization
AIP Conf. Proc. 2924, 040003-1–040003-5; https://doi.org/10.1063/5.0181655
Published by AIP Publishing. 978-0-7354-4852-0/$30.00

light conditions in the field, and the illumination intensity was 700 $\mu mol/m^2s$, which is normal for a clear day outside. The data for measuring the average daily illumination in the field for the last 10 years was taken from the NASA public service [10]. DRI-2000 and DM3-3000 lamps combined in equal proportions were used as the source of light.

FIGURE 1. Photograph of barley. (a) Two-row barley. (b) Six-row barley.

The air temperature in the vegetation chambers was maintained at 23±1ºC. Daytime lighting in the field test plot during the measurement period was in the range of 1400-1600 $\mu mol/m^2s$.

Chlorophyll fluorescence parameters of the flag leaves were measured in vivo using the LI-6800 photosynthesis system (LI-COR, USA) in a closed leaf chamber with a fluorimeter integrated in it. Measurements of the flag leaves of barley were performed at the completion stage of leaf blade formation. Temperature of 24ºC and a CO_2 concentration of 400 ppm was constantly maintained in the leaf chamber. To determine the maximum quantum yield of PSII (parameter F_v/F_m), the leaves were adapted to dark in the chamber of the device for about 20 minutes. We used the electron transport rate (ETR parameter) as a parameter that is connected to the structural and functional organization of the photosynthetic apparatus in thylakodine chloroplast membranes. To adapt the leaves to the light two levels of illumination inside the leaf chamber were used: 700 $\mu mol/m^2s$ for barley grown under controlled laboratory conditions and 1500 $\mu mol/m^2s$ for plants grown in the field.

RESULTS

Table 1 shows that the values of the maximum quantum yield of PS II (F_v/F_m) of the flag leaves of barley grown in light culture do not differ significantly from the values of the flag leaves of plants grown in the field. The maximum quantum yield of PS II in both samples was in the range of values typical for normal physiological state of plants [11]. At the same time, the speed of electronic transport in the plants grown in vegetation chamber was 1.7 times higher than in the field grown sample.

TABLE I. Comparison of the maximum quantum yield of PS II (F_v/F_m parameter) and the electron transport rate (ETR parameter) of the flag leaves of Emelya barley plants grown under controlled conditions of intensive light culture in a vegetation chamber and under natural conditions at the experimental field site.

	Vegetation chamber	Experimental field
F_v/F_m, relative units	0.79 ± 0.004	0.76 ± 0.002
ETR, $\mu mol * m^{-2} * s^{-1}$	124.3 ± 4.7	71.8 ± 9.1

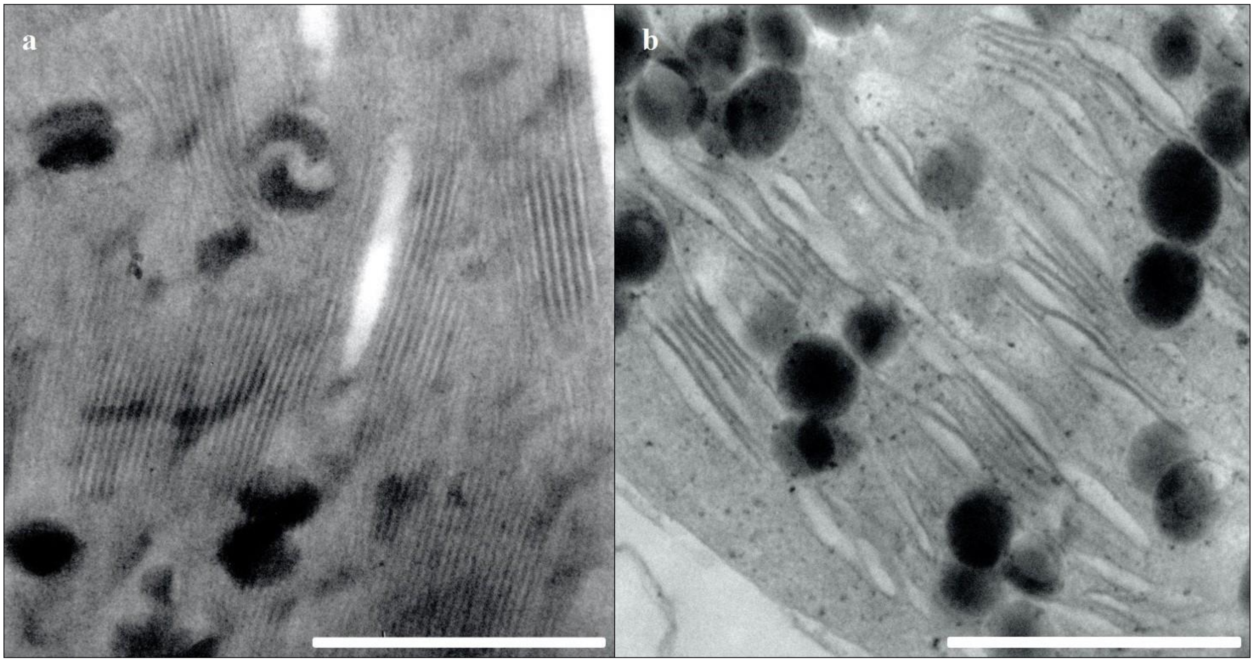

FIGURE 2. Transmission electron microscopy images of chloroplast in barley samples. Bar 500nm scale (a) Vegetation chamber. (b) Experimental field.

Such a big difference is well confirmed by the results of microscopy of tissue samples taken from the flag leaves of laboratory-grown barley plants and those grown under natural conditions in the field. Figure 2 presents a significantly denser and better ordered grana structure in the chloroplasts of barley leaf cells grown under light culture conditions. Since the transport of electrons is closely related to the spatial organization of the photosynthetic apparatus on the thylakoid membranes of chloroplasts, denser packed grana surely makes it possible to transport electrons more efficiently because molecular complexes involved in the process are better arranged.

DISCUSSION

Even Schrödinger [12] in his study was fair to name biological structures “aperiodic crystals”, thus confirming their high orderliness.

Studying the effect of chloroplast structure on optical properties has become more and more popular these days, although direct connection between a plant structure and optical properties has not yet been proved experimentally. Long-period ordering of structural elements that form layers with different permittivity has been discovered in plant leaves [9, 13].

Such structures are called photonic-crystal structures or biophotonic-crystal ones if we talk about biological objects. In the latter case, the ordering is hidden [9]. The spectrum of electromagnetic waves in such structures has bands.

The presence of photonic zones (stop bands) makes it possible to control the rate of optical radiation of atoms and molecules [14]. At the edges of the stop band the electromagnetic field is several times higher [15]. In this scenario, the structure may contain defects violating the photonic crystal structure periodicity, which leads to defect frequencies lying in the band gaps (the so-called defect modes). The density of photon states of these modes is much higher [15, 16]. Photonic crystals can be easily noted in the environment, for example, in butterfly wings [17, 18], fish scales [7], and chameleon skin [8]. Similar structures have been found in plant cells recently [19, 20, 21]. Any chloroplast has light-harvesting structures inside, so-called thylakoids (containing different systems, in particular photosynthetic ones). Thylakoids are collected in piles which are called grana. It has been discovered that optical properties of iridoplasts are determined by the way grana are arranged in them.

Radical changes in spectral characteristics of light waves propagating in the leaves of a plant surely affect the primary stages of photosynthesis.

In [22, 23] calculations were carried out to estimate the effect of a long-period structure on the optical properties and local characteristics of light waves propagating in a sample. Despite the fact that refractive indices in natural objects have a low contrast [24], the electromagnetic waves spectrum makes bands [25]. The density of photon states $\rho(\lambda)$ for this structure increases at the edges of a stop band. It was shown in [25] that the emission of atoms and molecules in the wavelength range from 460 to 560 nm is difficult. This is caused by the fact that molecules excited by light at a wavelength of 460 nm fluoresce in accordance with the Stokes rule at longer wavelengths falling into the stop band. Moreover, the absorption lines of chlorophylls a and b fall into the stop band too. In such cases, a strong interaction occurs between the exciton and Bragg resonances [26]. The red drop in the quantum yield is due to a decrease in the density of photon states in this wavelength area, like in Emerson enhancement. When molecules are irradiated with a wavelength in the red area, excited photons from the short-wavelength part of the spectrum are transported to absorption lines of chlorophylls. This leads to increase the quantum yield of photosynthesis in the red area of the spectrum.

In [22], the characteristics of transmission coefficients and field distribution in a finite layered-periodic structure were analyzed. The study shows that at the center of a structure the value of the electromagnetic field for the transmission maximum closest to the band gap can be more than an order of magnitude higher than the amplitude of the incident field. The maximum field strength increases linearly with an increase in the number of layers. The results were obtained for the ideal structure of a photonic crystal while in the work [4] calculations very close to the real structure of a wheat chloroplast were made. The study used a small number of layers and considered the dispersion from absorption lines of chlorophylls a and b. The results showed that such structures may have resonance effects and band gaps may be formed in the effective photosynthesis area. All these researches are prerequisites for considering the periodic structure of the chloroplast as a photonic crystal. The results of our work are in full agreement with this hypothesis.

CONCLUSION

The instrument data were related directly to grana ordering. This effect is explained not only by the density of packing and the convenience of transporting electrons, but also by resonance effects. Internal electromagnetic fields may play a significant role in the structure ordering. The thylakoids may move apart or push away from each other at extremely low or very high values, while pressing and holding together at moderate impact. Meanwhile, the ordering of the periodic structure has an impact on this very field. When percentage of layer packing gets higher, the refractive index increases, which in turn enhances the energy concentration in specific local zones and contributes to an increase in the quality factor. The concentration of hydrogen ions inside this structure also increases. High quality factors of the band gap in the area of efficient photosynthesis make it possible to perform photochemical reactions more efficiently [6]. Certainly, in addition to structural effects, there are also external influence factors, such as the intensity of incident light, which can also affect the internal structure of a leaf. Thus, the mechanism of rearrangement of thylakoid membranes improves the efficiency of photosynthesis, but protects itself from extremely high light exposure as well.

ACKNOWLEDGMENTS

The studies were performed on the equipment of Resource sharing center of the FRC KSC of the SB RAS.

REFERENCES

1. M. A. Korshunov, A. V. Shabanov, E. R. Bukhanov, and V. F. Shabanov, Dokl. Phys. **63**, 1–4 (2018).
2. A. Capretti, A. K. Ringsmuth, J. F. van Velzen, A. Rosnik, R. Croce, and T. Gregorkiewicz, Light Sci. Appl. **8**, 5 (2019).
3. M.-Y. Lin, W.-H. Xu, R. G. Bikbaev, J.-H. Yang, C.-R. Li, I. V. Timofeev, W. Lee, and K.-P. Chen, Materials **14**, 2788 (2021).
4. E. Bukhanov, A. V. Shabanov, M. N. Volochaev, and S. A. Pyatina, Plants **10**, 1967 (2021).
5. H. T. Witt, *Primary acts of Energy Conservation in the Functional Membrane of Photosynthesis; Bioenergetics of Photosynthesis/ed. by Govindjee* (Academic Press: New York, NY, USA, 1975).
6. H. T. Witt, Biochim. Biophys. Acta. **505**, 355–427 (1979).
7. D. R. McKenzie, Y. Yin, and W. D. McFall, Proc. R. Soc. Lond. A **451**, 579–584 (1995).
8. J. Teyssier, S. V. Saenko, D. van der Marel, and M. C. Milinkovitch, Nat. Commun. **6**, 6368 (2015).
9. M. Jacobs, M. Lopez-Garcia, O. P. Phrathep, T. Lawson, R. Oulton, and H. M. Whitney, Nat. Plants. **2**, 16162 (2016).
10. P. data access viewer, *https://power.larc.nasa.gov/data-access-viewer/* (Web-site, 2022).
11. H. K. Lichtenthaler, C. Buschmann, and M. Knapp, Photosynthetica **43**, 379–393 (2005).
12. E. Schrödinger, *What is Life? The Physical Aspect of the Living Cell* (Cambridge University Press, 1944).
13. S. Vignolini, E. Moyroud, B. J. Glover, and U. Steiner, J. R. Soc. Interface **10**, 20130394 (2013).
14. V. P. Bykov, Soviet Jour. of Quan. Electr. **4(7)**, 861–866 (1975).
15. V. S. Gorelik and V. V. Kapaev, JETP **123(3)**, 373–381 (2016).
16. V. F. Shabanov, S. Y. Vetrov, and A. V. Shabanov, *Optics of Real Photonic Crystals* (SB RAS Publisher, 2005).
17. S. Kinoshita, Rep. on Prog. in Phys. **71(7)**, 076401 (2008).
18. P. Vukusic and J. R. Sambles, Nature **424**, 852–855 (2003).
19. J. W. Liu and et. al., Am. J. Bot. **107**, 562–576 (2020).
20. R. Ghaffar, M. Weidinger, B. Mähnert, M. Schagerl, and I. Lichtscheidl, Plant Cell Environ. **41**, 1791–1805 (2018).
21. S. H. Pao and et al., J. Plant Res. **131**, 655–670 (2018).
22. A. V. Shabanov, M. A. Korshunov, and E. R. Bukhanov, Comp. Opt. **41**, 680–686 (2017).
23. A. V. Shabanov, M. A. Korshunov, and E. R. Bukhanov, Comp. Opt. **43(2)**, 231–237 (2019).
24. G. Paillotin, Biophys. **75**, 124–133 (1998).
25. Kirchhoff, Proc. Nat. Acad. USA. **108(50)**, 20249–20253 (2011).
26. D. R. Kazanov, A. V. Poshakinskiy, and T. V. Shubina, Jetp Lett. **105**, 8–12 (2017).