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# Assembly of Pigment–Protein Complexes in Carotenoid-Deficient Membranes of Plastids from Wheat Seedlings Treated with Norflurazon

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**Abstract**—The pyridazinone-type herbicide norflurazon SAN 9789 inhibiting the biosynthesis of long-chain carotenoids results in significant decrease in PS II core complexes and content of light-harvesting complex (LHC) polypeptides. At the same time, early light-induced proteins (ELIP) with molecular masses of 20.5-16.5 and 13.5 kD disappear in norflurazon-treated seedlings grown under intermittent (pulsed) light, confirming the hypothesis that they are carotenoid-binding proteins. Full disappearance of Chl *a* forms at 668, 676, and 690 nm and a sharp decrease in Chl *b* form at 648 nm in treated seedlings grown under 30 or 100 lx light intensity shows close contact of these forms with carotenoids in the thylakoid membrane. The band shift from 740 to 720 nm in the low-temperature fluorescence spectrum (77 K) suggests a disturbance of energy transfer from LHC to the Chl *a* form at 710-712 nm.

*Key words*: carotenoids, chloroplasts, light-harvesting complex, fluorescence, wheat

Photosynthetic membrane of chloroplasts of higher plants and green algae collect light energy through an antenna system consisting of pigment–protein complexes. The complexes contain chlorophyll (Chl) and various carotenoids (Car) with different cyclic end-groups [1-3]. The absorbed energy is transferred to the reaction centers (RC) of photosystem (PS) I and PS II to drive primary charge separation and electron transfer through an electron-transport chain.

Carotenoids are a large class of natural pigments that differ in structure and function [4, 5]. At present more than 600 structurally different carotenoids are known [6]. They are synthesized in almost all prokaryotic and eukaryotic organisms and are an essential component of photosynthetic membranes. They are present in protein complexes in the thylakoid membrane and play multiple roles in photosynthesis. According to contemporary data, carotenoids contribute to harvesting and transfer of light energy, maintain the function of reaction centers and light-harvesting complexes (LHC) of the photosystems, as well as provide protective functions effectively by quenching Chl triplet states, scavenging singlet oxygen species, and dissipating excess energy absorbed by antenna chlorophyll [2, 3, 5, 7, 8].

In recent years the study of carotenoids has sharply increased in connection with new data on antioxidant activity of carotenes and xanthophylls [9], detection of possible participation of xanthophyll via the violaxanthin cycle in the formation and function of pigment–protein complex of PS II [7], and its possible localization near PS I [10]. Work [11] showed that a definite ratio of Chl *b* and antera- and violaxanthin in the pigment ensemble is needed for the formation of normal thylakoid grana.

Carotenoids, especially xanthophylls, protect the native form of chlorophylls from destructive photooxidation and stabilize the structure of pigment–protein complexes in thylakoid membranes [8, 12]. It was established that whereas β-carotene is mainly localized in the core complexes of PS I and II, the xanthophylls violaxanthin and neoxanthin as well as lutein are predominantly found in light-harvesting complexes [13]. The formation of zeaxanthin from violaxanthin under high light stress via the so-called xanthophyll cycle is a well-described phenomenon [7].

It has been shown that violaxanthin is present in substoichiometric amounts in light harvesting Chl *a*/*b*-binding protein complex of PS II (LHC II) on the peripheral part of the complex where it is supposed to play an important structural role to control LHC II macroorganization [14]. It was also confirmed that the core of PS II (CP47

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and CP43) binds not only β-carotene, but also lutein [15] and minor Chl *a*/*b*-proteins (CP24, CP26, and CP29) that regulate energy transfer to the RC. It is supposed that zeaxanthin joins predominantly to several minor components of LHC II—stress-induced proteins (ELIP, early light-induced proteins)—after long exposure to high light illumination [16-18]. The release of violaxanthin from LHC II and its partial rearrangement caused by zeaxanthin influences the structure and conformation of light-harvesting complexes and maintain light-induced changes in the major and minor LHC, which results in energy quenching [14]. The structural function of lutein for oligomerization of light-harvesting complexes during assembly of the photosynthetic apparatus has also been determined [19]. Although less is known about the Car composition of light-harvesting proteins associated with LHC I, all LHC proteins are believed to contain at least three Car binding sites, which show preferential binding for the xanthophylls lutein, violaxanthin, and neoxanthin [20].

Thus, despite intensive studies in the field of carotenoids of higher plants and algae, their role and function in light-harvesting and light dissipation has not been studied in detail. Wheat seedlings grown in darkness and at low light intensity in the presence of norflurazon, an inhibitor of carotenoid synthesis, were used for partial solution of this problem. It should be noted that norflurazon is not a unique suppresser of carotenoid biosynthesis. A similar effect is achieved in bacteria using diphenylamine [21].

## MATERIALS AND METHODS

The studies focused on a local hard winter variety of wheat (*Triticum durum* L.) Garagylchyg-2 developed at the Azerbaijan Scientific-Research Institute of Agriculture. Seeds were wetted with water in 2-3 h and then were transferred to Whatman 3MM filter paper. Seedlings were germinated and grown in aqueous culture in a growth chamber at 23 ± 2°C and 50-60% relative humidity in darkness or in continuous illumination intensity 130 and 300 mW/m$^2$, which corresponds to 30 and 100 lx intensity ("white" luminescent lamps LB-40 W; Razno, Russia). The herbicide norflurazon (a derivative of pyrazon)—4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2H)-pyridazinone (SAN 9789, Sandoz)—was used as an inhibitor. Seedlings were grown in the presence or absence of norflurazon (control). The treatment of seedlings by herbicide was carried out following the method as described in [22]. Pyridazine SAN 9789 was dissolved in 96% ethanol and transferred onto a double layer of a filter paper in a Petri dish (500 mg/m$^2$). After complete evaporation of ethanol, 10 ml of water was added (final norflurazon concentration corresponds to 50 μM).

Leaves were mixed with chilled grinding buffer (0.4 M sucrose, 10 mM Tricine-NaOH, pH 7.6, 10 mM $MgCl_2$) and homogenized for 5 sec (4°C) at full power in a MPW-302 blender (Mechanika Precyzyja, Poland). Chloroplast isolation and thylakoid membrane precipitation were carried out according to Peter and Thornber [23]. The chloroplast suspension was centrifuged at 1000*g* for 10 min at 4°C in a K-70 centrifuge (VEB MLW Medizintechik Leipzig, Germany; rotor radius $r = 8$ cm) and the chloroplast pellet was resuspended in lysis buffer (10 mM Tricine-NaOH, pH 7.6, 2 mM Na-EDTA) with a glass homogenizer. The homogenate was centrifuged at 27,000*g* (Beckman L-90K ultracentrifuge, USA; rotor radius $r = 16$ cm) for 5 min at 4°C. The chlorophyll concentration was determined spectrophotometrically in 80% acetone extract [24]. Carotenoids in total extracts were determined according to the formula of Wettstein [25].

The polypeptide composition of samples was analyzed by SDS-PAGE according to Laemmli [26] using a 6% stacking gel and a 10 to 25% acrylamide gradient in the separating gel as described in [27]. The gels were 1 mm thick, 16 cm wide, and 18 cm long. After electrophoresis, the gels were stained for 30 min with a solution of 0.04% Coomassie Brilliant Blue G-250 prepared in 3.5% perchloric acid ($HClO_4$). The gels were scanned using an ULTROSCAN 2202 densitometer (LKB, Sweden) with a 633 nm laser as the light source. A set of standard proteins (Sigma, USA) was used for determination of molecular mass of polypeptides.

The spectral measurements at 77 K were performed using a double-beam Hitachi-557 (Japan) spectrophotometer and a Hitachi-850 (Japan) fluorescence spectrophotometer as described previously [28]. Fluorescence emission spectra were corrected for the spectral sensitivity of the spectrofluorimeter using rhodamine B.

## RESULTS AND DISCUSSION

It should be noted that wheat seedlings grown at 30 and 100 lx light intensity in the presence of the herbicide norflurazon do not differ from control plants in size. As a result of blocking carotinoid biosynthesis of treated seedlings, light green color is observed, which is strongly expressed at 100 lx illumination. The seedlings illuminated after dark incubation with herbicide within 5-6 days grow the same way as untreated cultures, although the content of carotenoids in them is significantly low. Apparently, the applied concentration of norflurazon (50 μM) does not influence growth of the culture. Similar data are obtained in the study of pyridazinone herbicide effect on the cell growth of *Cyanidium caldarium* [29]. It was shown that upon transfer of herbicide treated seedlings grown in intermittent (pulsed) illumination to continuous illumination the characteristic light-green color turns to white. Obviously, the blocking of

carotenoids synthesis causes the formation of photolabile pigment apparatus in leaves, which is destroyed under the influence of light.

Figure 1 presents data related to the content of photosynthetic pigments in control and norflurazon-treated seedlings grown under different light regimes. In control plants with increasing light intensity the content of chlorophyll *a* and *b* and carotenoids increases, while the Chl *a*/*b* ratio decreases. These results show intensification of pigment biosynthesis during biogenesis of the photosynthetic apparatus under intense illumination. However, when wheat seedlings are grown in the presence of norflurazon dependence of the pigment content on illumination intensity has a completely different effect. Under low-light illumination (30 lx), the seedlings contain a significant amount of Chl *a*, but lower amount of Chl *b*. The decrease in carotenoid content in treated seedlings is accompanied by decrease in chlorophyll levels. Increasing the light intensity to 100 lx for norflurazon-grown plants leads to a sharp decrease in the content of all photosynthetic pigments (Fig. 1). Since Chl *b* exists predominantly in light-harvesting chlorophyll *a*/*b*-complex, the reduced content of this pigment is explained by a low LHC content in the treated seedlings.

The densitometry of high-resolution gradient (10-25%) electrophoregrams is present in Fig. 2. More than 25 bands in the region from 69 to 11 kD are observed in chloroplast membranes of control plants that have been grown at 100 lx illumination. When comparing of profile of distribution of thylakoid membrane polypeptides of control and treated plants, significant differences were detected in their protein composition. The content of the apoprotein of PS I core with molecular mass of 69 kD considerably decreases in treated plants. In seedlings illuminated at 100 lx, the 47-kD band disappears completely, and trace amounts of 51 and 43 kD polypeptides that are included in the composition of core antenna of PS II [30] are seen. The changes observed in the 29.5-21 kD polypeptide region are due to light-harvesting Chl *a*/*b*-protein complexes LHC I and II. After norflurazon treatment, some of these proteins disappear completely, but only notable decrease in the amount of protein is observed in others (especially in seedlings grown at 100 lx). The most widely accepted suggestion is that functional chloroplasts produce a positive signal, the "plastidic factor", required for the expression of nuclear genes for chloroplast proteins, and that production of this factor is prevented by photooxidation of chloroplasts [31]. Significant changes are also observed in the content of 37.5, 36, 22, 21, 16.5, 13.5, and 11 kD polypeptides; the last two completely disappear in seedlings grown at 100 lx. The content of 15 kD polypeptide increases in herbicide-treated seedlings. We previously found a similar polypeptide in a study on formation of photosynthetic apparatus in wheat seedlings during greening [17, 18]. The 22 kD polypeptide (product of the *PsbS* gene) revealed on the densitogram, which belongs to PS II, was also detected in cyanobacterium and barley, which demonstrates its homology to LHC proteins and ELIP (early light-induced proteins), and, in the opinion of the authors of [32] does not bind Chl. But recently it was found that PsbS is a Chl *a*/*b*-binding subunit of PS II and has a small amount of bound carotenoids [33].

We focused our interest on a set of bands in SDS-PAGE in the 29.5-21 kD region from seedlings grown at 30 lx intensity, which relates to light-harvesting Chl *a*/*b*-protein complexes LHC I and II. According to data obtained by us earlier, these bands consist of the monomeric forms of light-harvesting complexes [17, 18]. After further illumination, the number of these bands decreases on the densitogram and the picture characteristic for mature chloroplasts becomes clear. The decrease in band number in the LHC region evidently confirms the fact that monomeric forms of LHC aggregate to form higher-order oligomeric forms. Similar results were observed in [19] on reconstruction of Chl *a*/*b*-binding light-harvesting complexes.

As seen in the densitogram, the intensity of the 13.5 kD band drops sharply in treated samples (especially in seedlings grown at 100 lx light intensity). This polypeptide may be a product of the *LHCIIe* gene, which binds high levels of carotenoids, especially violaxanthin [34], and the high xanthophyll content provides a photoprotective function of the *LHCIIe* gene product. The

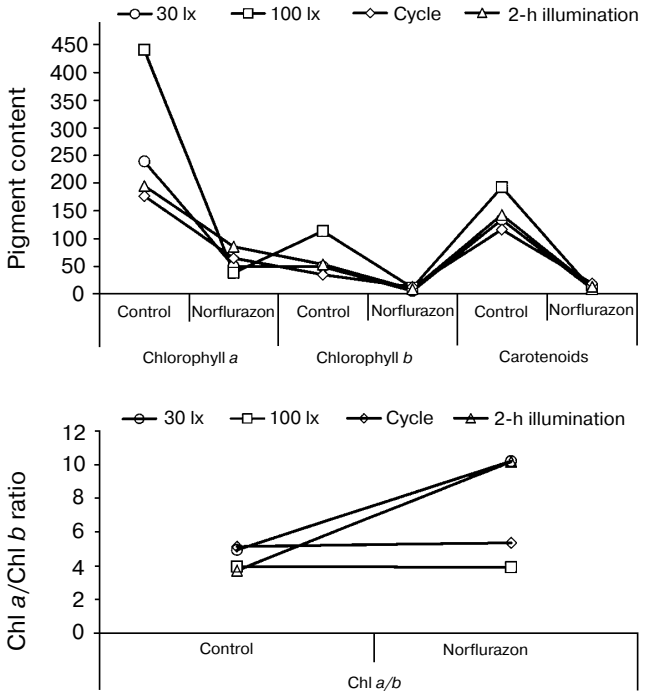


**Fig. 1.** Pigment content (μg/g wet tissue weight) of wheat seedlings in the absence or presence of norflurazon grown under various illuminations.

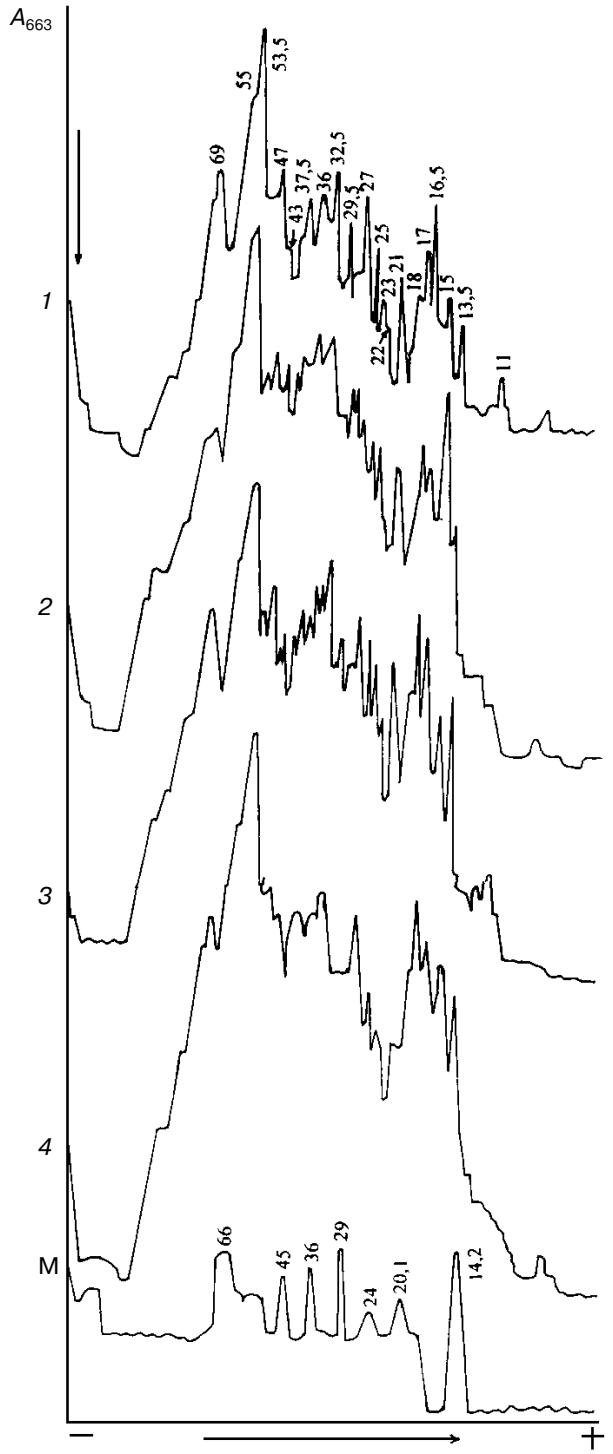


**Fig. 2.** Density patterns from gradient (10-25%) SDS-PAGE analysis of thylakoid membranes of wheat seedlings grown under 100 and 30 lx light intensity in the presence (*2*, *4*) or absence of norflurazon (*1*, *3*). M, standard proteins (in kD): bovine serum albumin (66), ovalbumin (45), glyceraldehydes-3-phosphate dehydrogenase (36), carbonic anhydrase (29), trypsinogen (24), trypsin inhibitor (20.1), lactalbumin (14.2). Electrophoresis was carried out in Tris-glycine buffer, pH 8.3, at 4°C for 16 h.

content of 25 kD polypeptide binding chlorophyll *b* also decreases in wheat seedlings [35].

For seedlings grown in intermittent (pulsed) light the electrophoregram shows polypeptides of 20.5-16.5 and 13.5 kD. According to the contemporary literature, these are related to "early light-induced proteins" (ELIP) [36-38] (data not shown). ELIP are products of the *cbr* gene, being fractionated together with LHC II and induced by β-carotene accumulation under extreme conditions. No definite nature and function have yet been finally described for ELIPs in higher plants. After treatment with norflurazon, these proteins either disappear or their content decreases significantly. The results suggest that early light-induced proteins are carotenoid-binding proteins.

Thus, the blocking of carotenoid biosynthesis in plants exerts a harmful effect on thylakoid membrane proteins making them more responsive to proteolytic degradation. Probably one of the causes of gene expression inhibition may be destruction by photooxidation of chloroplast signals originating in the chloroplast targeted to the nucleus and needed for optimal expression of nuclear genes encoding chloroplast proteins [31, 39]. As a result, synthesis of subunits related to light-harvesting Chl *a*/*b*-protein complexes encoded in the nucleus is impeded (see Fig. 2).

Blocking of carotenoid synthesis by norflurazon also results in changes in spectral characteristics and native state of chlorophyll of leaves.

Figures 3 and 4 show absorption spectra (*A*) and their fourth derivatives ($A^{IV}$) at 77 K of seedlings from the control and herbicide-treated plants grown at 30 lx illumination. The absorption spectrum from control seedlings is characterized by main band of Chl *a* at 676 nm in the red region and Chl *b* at 650 nm. In the fourth derivative of the absorption spectra at 77 K are localized Chl *b* form at 648 nm and Chl *a* forms at 660, 668, 676, 682, 690, 696, 704, and 712 nm. There are significant changes in the organization of pigment systems in norflurazon-treated plants (Fig. 4). The Chl *a* forms at 668, 676, and 690 nm that belong to the composition of the LHC and antenna part of PS I disappear after treatment. The special form of Chl *a* at 637 nm is also completely absent in treated seedlings, and the intensity of the Chl *b* band at 648 nm is sharply decreased compared to the control. At the same time, as shown in Fig. 4, the intensities of the Chl *a* forms at 660 and 696 nm increase and the intensity of Chl *a* at 704 nm, which belongs to the reaction center of PS I, decreases noticeably. The significant changes in the native organization of chlorophylls apparently show the existence of close contact of these forms with carotenoids in the formation and organization of the photosynthetic apparatus. It is interesting to note that there is no noticeable change in the intensity of the Chl *a* peak at 682 nm in this case (together with the content of 32.5 kD protein due to the RS of PS II).

Comparative analysis of the fourth derivatives of the absorption spectra at 77 K of control and norflurazon-treated seedlings in the blue region also shows significant changes: the intensity of the absorption spectrum sharply decreases in the region of carotenoids (400-500 nm) and the β-carotene form at 496 nm disappears. The bands of carotenoid absorption at 480, 492, and 508 nm also disappear. It was suggested that apart from a protective role, the form at 496 nm also plays a structural role as a binding link between PS II RC and LHC II simultaneously in the center of the whole PS II assembly. These results coincide with the data [12] that also prove the necessity of carotenoids for the assembly of active PS II. It was also suggested that the presence of minimum contents of β-carotene hydroxylases and minimum contents of specific β-carotene-binding xanthophylls is necessary for assembly and function of LHC II [40]. The obvious decrease in the ratio of peak amplitudes of Chl *b* at 472 nm appear for the treated chloroplasts in comparison with control, which corresponds to the data in the red region of the spectrum. Similar results are obtained in seedlings grown at 100 lx irradiance in the presence of norflurazon.

In the low temperature fluorescence spectra (77 K) of the control seedlings there are bands at 687, 695 (shoulder), and 740-742 nm, which are attributed to core antenna of PS II and peripheral antenna of PS I, accordingly (Fig. 5). In seedlings greening at 30 and 100 lx light intensity with norflurazon remarkable changes are observed in the fluorescence spectrum: there is a short wavelength shift of the main maximum from 740 to 720 nm and its intensity decreases. At the same time redistribution of bands and increase in fluorescence at 687 and 695 nm regions are observed. According to the data obtained by us on the contents of pigments (shown above), in seedlings treated by the herbicide a short wavelength shift of the maximum in the fluorescence spectra from 740 to 720 nm is coupled with a decrease in the amount of Chl *a* and *b* in PS I antenna. A similar spectral shift was attributed to the removal of chlorophyll from the so-called peripheral antenna [17, 18].

The Chl *a* form at 710-712 nm is known to be responsible for the long-wavelength fluorescence band of seedlings at 740 nm. According to the date of the fourth derivative absorption spectra at 77 K, the long wavelength

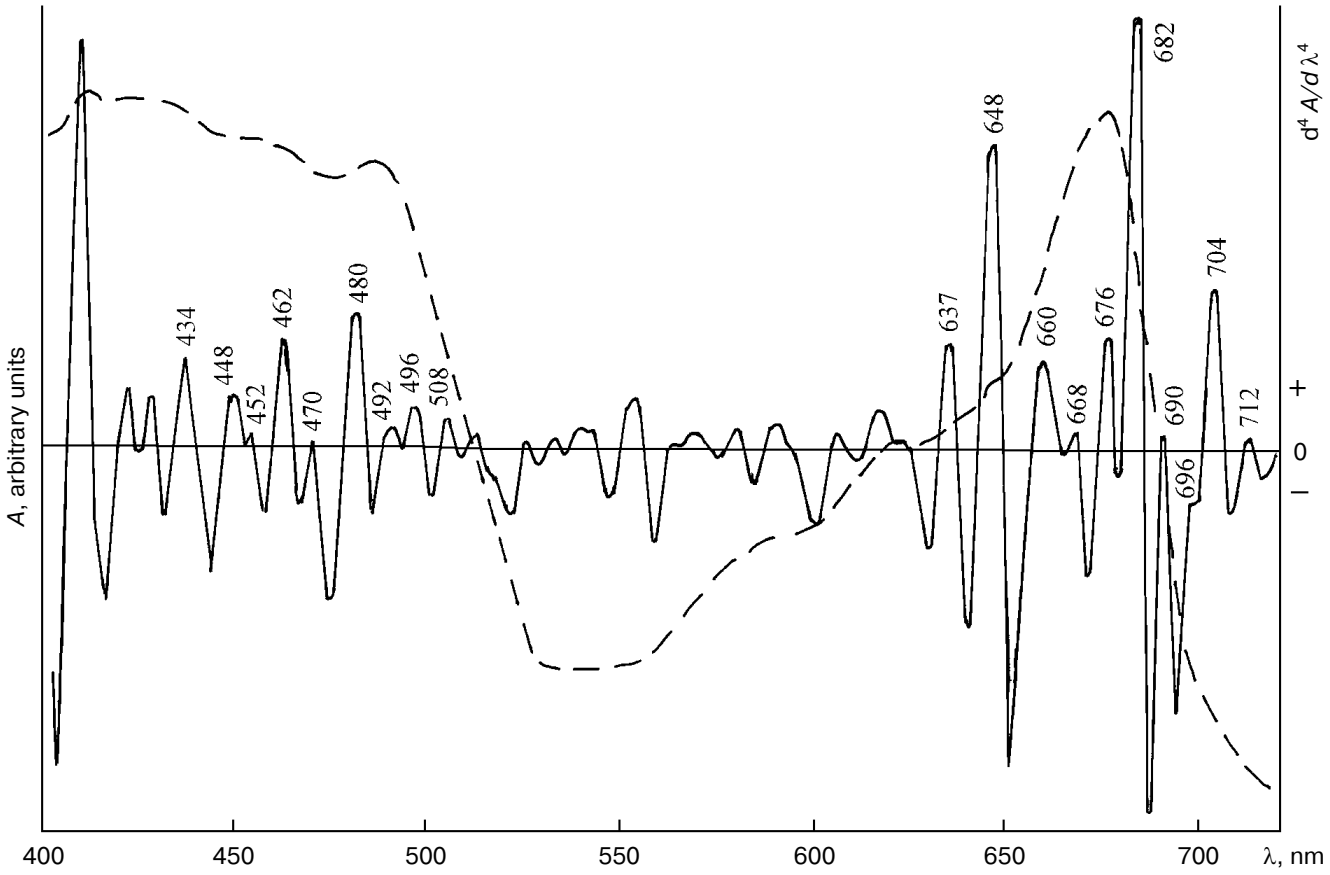


**Fig. 3.** Absorption spectra (dashed line) and fourth derivatives (solid line) at 77 K of control wheat seedlings grown under 30 lx light intensity.

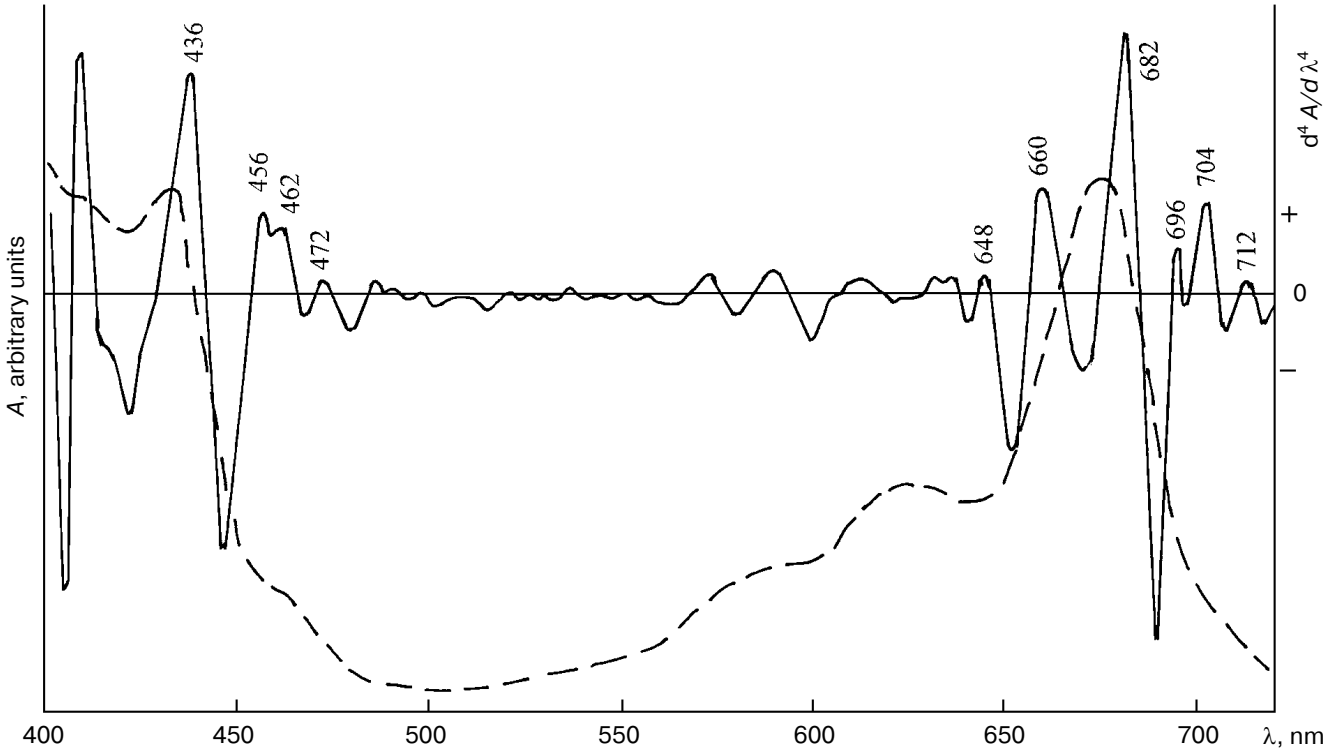


**Fig. 4.** Absorption spectra (dashed line) and fourth derivatives of the absorption spectra (solid line) at 77 K of wheat seedlings grown under 30 lx light intensity in the presence of norflurazon.

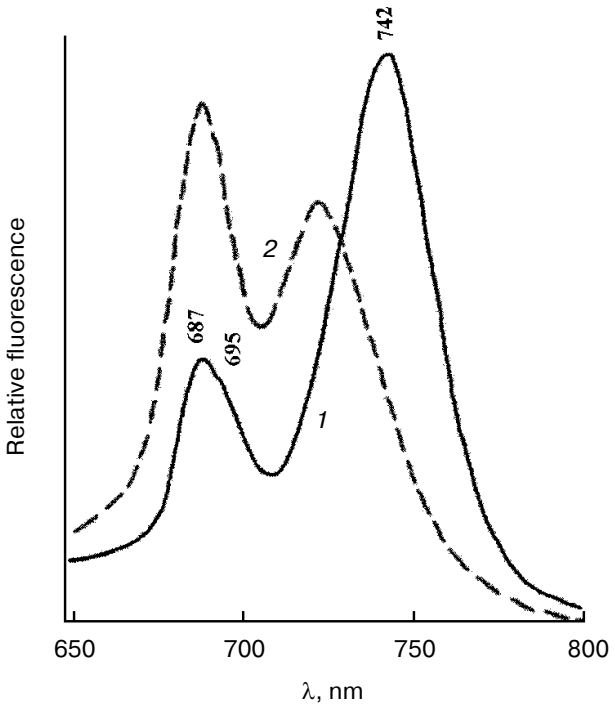


**Fig. 5.** Fluorescence emission spectra at 77 K obtained from wheat seedlings grown under 30 lx light intensity in the absence (*1*) or presence of norflurazon (*2*).

Chl *a* forms are also present in treated leaves (Fig. 4). Therefore, the shift to shorter wavelength with the large decrease in the intensity of the long wavelength fluorescence band of the seedlings treated by norflurazon cannot be explained by the absence of the longest wavelength Chl *a* forms. Seedlings with blocking of carotenoid synthesis most probably lack energy transfer from the light-harvesting complex to the Chl *a* form at 710-712 nm and, therefore, this form fluoresces weakly.

So, numerous structural and functional disturbances shown in pyridazinone-treated plants are direct results of photodestructive processes in the absence of carotenoids. The decrease in the size in the light-harvesting complex of PS II (LHC II) under treatment promotes photoprotection by decrease in light absorption and generation of $^1O_2^*$ in the lipid phase of the membrane [41]. Our results also indicate important significance of carotenoids not only in functioning of PS I and II, but also in structural organization of the chloroplast membrane. Thus, in the plants grown with norflurazon in the greening process there is a disturbance of regulatory mechanisms controlling chloroplast development. It is possible that carotenoids, in addition to a structural role, also play a stabilizing role in the formation of pigment–protein complexes of the wheat photosynthetic membrane.

## REFERENCES


1. Cunningham, F., and Gantt, E. (1998) *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **49**, 557-583.
2. Ladygin, V. G. (2000) *Fiziol. Rast.*, **47**, 904-923.
3. Ladydin, V. G. (2001) *Uspekhi Sovrem. Biol.*, **121**, 296-317.
4. Britton, V. G. (1986) *Biochemistry of Natural Pigments* [Russian translation], Mir, Moscow.
5. Britton, G. (1995) *FASEB J.*, **9**, 1551-1558.
6. Straub, O. (1987) in *Key to Carotenoids* (Plander, H., ed.) 2nd Edn., Birkhauser Verlag, Basel, pp. 11-296.
7. Demmig-Adams, B., Gilmore, A. M., and Adams, W. W. (1996) *FASEB J.*, **10**, 403-412.
8. Moskalenko, A. A., and Karapetyan, N. V. (1996) *Z. Naturforsch.*, **51**, 763-771.
9. Miller, N. J., Sampson, J., Candlias, L. P., Bramley, P. M., and Rice-Evans, C. A. (1996) *FEBS Lett.*, **384**, 240-242.
10. Ladygin, V. G., and Shirshikova, G. N. (1987) *Fiziol. Rast.*, **34**, 1068-1072.
11. Paxlavuni, I. K., Chibullina, L. A., and Serebryakova, V. N. (2000) *Fiziol. Rast.*, **47**, 43-52.
12. Humbeck, K., Romer, S., and Senger, H. (1989) *Planta*, **179**, 242-250.
13. Lee, A., and Thornber, J. P. (1995) *Plant Physiol.*, **107**, 565-574.
14. Horton, P., Ruban, A. V., and Walters, R. G. (1996) *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **47**, 675-684.
15. Bassi, R., Pincau, B., Dainese, P., and Marquardt, J. (1993) *Eur. J. Biochem.*, **212**, 297-303.
16. Krol, M., Spangfort, M. D., Huner, N. P. A., Oquist, G., Gustafsson, P., and Jansson, S. (1995) *Plant Physiol.*, **107**, 873-883.
17. Guseinova, I. M., Suleimanov, S. Y., Zulfugarov, I. S., and Aliyev, J. A. (2000) *J. Fluoresc.*, **10**, 255-259.
18. Aliyev, J. A., Guseinova, Y. M., Suleimanov, S. Yu., and Zulfugarov, I. S. (2001) *Biochemistry (Moscow)*, **66**, 490-495.
19. Romer, S. (1999) in *The Chloroplast: From Molecular Biology to Biotechnology* (Arguroudi-Akoyunoglou, J. H., and Senger, H., eds.) Kluwer Academic Publishers, Dordrecht-Boston-London, pp. 217-223.
20. Aro, E.-M., Virgin, I., and Andersson, B. (1993) *Biochim. Biophys. Acta*, **1143**, 113-134.
21. Krikunova, M. A., Leupold, D., Rini, M., Voigt, B., Moskalenko, A. A., Toropygina, O. A., and Razzhivin, A. P. (2002) *Biofizika*, **47**, 1015-1020.
22. Bolychevtseva, Yu. V., Chivkunova, O. B., Merzlyak, M. N., and Karapetyan, N. V. (1987) *Biokhimiya*, **52**, 160-167.
23. Peter, G. F., and Thornber, J. P. (1991) *J. Biol. Chem.*, **266**, 16745-16754.
24. Mc-Kinney, G. (1941) *J. Biol. Chem.*, **140**, 315-322.
25. Wettstein, D. (1957) *Exp. Cell Res.*, **12**, 427-436.
26. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
27. Aliyev, J. A., Suleimanov, S. Yu., Guseinova, I. M., Asadov, A. A., and Ismailov, M. A. (1992) *Biokhimiya*, **57**, 679-686.
28. Asadov, A. A., Zulfugarov, I. S., Suleimanov, S. Yu., and Alyiev, J. A. (1986) *Dokl. AN SSSR*, **287**, 444-447.
29. Yurina, N. P., Karakashev, G. V., Odintsova, M. S., and Karapetyan, N. V. (1989) *Dokl. AN SSSR*, **306**, 739-742.
30. Dreyfuss, B. W., and Thornber, J. P. (1994) *Plant Physiol.*, **106**, 829-839.
31. Strand, A., Asami, T., Alonso, J., Esker, J. R., and Chory, J. (2003) *Nature*, **421**, 79-82.
32. Kim, S., Sandusky, P., Bowbly, N. R., Aebersold, R., Green, B. R., Vlahakis, S., Yocum, C. F., and Pichersky, E. (1992) *FEBS Lett.*, **314**, 67-71.
33. Funk, C., Schroder, W. P., Green, B. R., Renger, G., and Andersson, B. (1994) *FEBS Lett.*, **342**, 261-266.
34. Irrgang, K. D., Kablitz, B., Vater, J., and Renger, G. (1993) *Biochim. Biophys. Acta*, **1143**, 173-182.
35. Rurie, I. J., Anderson, J. M., and Goodchild, D. J. (1980) *Eur. J. Biochem.*, **107**, 345-354.
36. Adamska, I., Kloppstech, K., and Ohad, I. (1993) *J. Biol. Chem.*, **268**, 5438-5444.
37. Adamska, I., Roobo-Boza, M., Lindahl, M., and Andersson, B. (1999) *Eur. J. Biochem.*, **260**, 453-460.
38. Harari-Steinberg, O., Ohad, I., and Chamovitz, D. A. (2001) *Plant Physiol.*, **127**, 986-997.
39. Taylor, W. C. (1989) *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **40**, 211-233.
40. Tian, L., Margallanes-Lundback, M., Musetti, V., and Della Penna, D. (2003) *The Plant Cell*, **15**, 1320-1332.
41. Baroli, I., Do, A. D., Yamane, T., and Niyogi, K. K. (2003) *The Plant Cell*, **15**, 992-1008.