AGRICULTURAL BIOLOGY,
2018, V. 53, № 1, pp. 38-49
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Agrobiology of grain crops

UDC 631.8:546.26:581.1

doi: 10.15389/agrobiology.2018.1.38eng
doi: 10.15389/agrobiology.2018.1.38rus

FULLERENE DERIVATIVES INFLUENCE PRODUCTION PROCESS, GROWTH AND RESISTANCE TO OXIDATIVE STRESS IN BARLEY AND WHEAT PLANTS

G.G. PANOVA[1], E.V. KANASH[1], K.N. SEMENOV[2], N.A. CHARYKOV[3], Yu.V. KHOMYAKOV[1], L.M. ANIKINA[1], A.M. ARTEM'EVA[4], D.L. KORNYUKHIN[4], V.E. VERTEBNYI[1], N.G. SINYAVINA[1], O.R. UDALOVA[1], N.A. KULENOVA[5], S.Yu. BLOKHINA[1]

[1]*Agrophysical Research Institute*, Federal Agency for Scientific Organizations, 14, Grazhdanskii prosp., St. Petersburg, 195220 Russia, e-mail gaiane@inbox.ru (✉ corresponding author), ykanash@yandex.ru, himlabafi@yandex.ru, lanikina@yandex.ru, verteb22@mail.ru, sinad@inbox.ru, udal59@inbox.ru, sblokhina@agrophys.ru;
[2]*Saint Petersburg State University,* 26, Universitetskii pr., St. Petersburg—Petrodvorets, 198504 Russia, e-mail k.semenov@spbu.ru;
[3]*Saint-Petersburg State Institute of Technology (Technical University),* 26, Moskovskii pr., St. Petersburg, 190013 Russia, e-mail ncharykov@yandex.ru;
[4]*Federal Research Center the Vavilov All-Russian Institute of Plant Genetic Resources,* Federal Agency for Scientific Organizations, 42-44, ul. Bol'shaya Morskaya, St. Petersburg, 190000 Russia, e-mail akme11@yandex.ru, dkor4@yandex.ru;
[5]*Serikbayev East Kazakhstan State Technical University,* 69, ul. A.K. Protozanova, Ust-Kamenogorsk, 070004 Republic of Kazakhstan, e-mail nkulenova@ektu.kz
ORCID:
Panova G.G. orcid.org/0000-0002-1132-9915
Kanash E.V. orcid.org/0000-0002-8214-8193
Semenov K.N. orcid.org/0000-0003-2239-2044
Charykov N.A. orcid.org/0000-0002-4744-7083
Khomyakov Yu.V. orcid.org/0000-0003-3245-8801
Anikina L.M. orcid.org/0000-0001-5217-174X
Artemyeva A.M. orcid.org/0000-0002-6551-5203
Kornyukhin D.L. orcid.org/0000-0001-9181-5368
Vertebnyi V.E. orcid.org/0000-0002-7817-2721
Sinyavina N.G. orcid.org/0000-0003-0378-7331
Udalova O.R. orcid.org/0000-0003-3521-0254
Kulenova N.A. orcid.org/0000-0002-7063-4899
Blokhina S.Yu. orcid.org/0000-0003-3492-6808
The authors declare no conflict of interests
Acknowledgements:
Supported financially by Russian Foundation for Basic Research (project № 15-29-05837 ofi_m)
Received March 28, 2017

Abstract

Creation of effective environment-friendly preparations to improve productivity and sustainability of agro- and ecosystems is of current interest. Carbon nanostructures, such as the water-soluble C_{60} and C_{70} fullerene derivatives presently used in biomedicine and pharmacology, are considered perspective agents for agriculture. It was shown that they can penetrate into the cell membranes owing to lipophylicity and nanosize, transport medicinal substances to target cells and have antioxidant activity. The mechanism underlying the influence of water-soluble fullerene derivatives on plants in agroecosystems remains unclear. In the paper, we for the first time report the effects of C_{60} fullerene derivatives on the processes that determine the net productivity and plant resistance to oxidative stress. In the study we used fullerenol and the fullerene C_{60} adducts with the three essential amino acids, threonine, lysine, arginine, and also with the amino acid hydroxyproline, which were previously synthesized following a one-step procedure. Stimulating effects of these fullerene derivatives on the growth of spring wheat and barley were observed in two vegetation experiments carried out in controlled conditions (aerated nutrient solution, plant growing light equipment) when the compounds were added to the root habited medium and under non-root treatment. It was shown that the biomass of leaves, stems, and roots in plants increased by 27-226 % ($p < 0.05$). Statistical analysis using the Wilcoxon test confirmed the reliability of the differences found. Fullerenol, fullerene C_{60}-hydroxyproline, and fullerene C_{60}-threonine caused the greatest increase when compared to the control. Obviously, the observed effect was associated with the established ability of fullerenol and C_{60} fullerene amino acid derivatives to exert regulatory activity on the synthesis of photosynthetic pigments and, as a consequence, on the efficiency of photosynthesis. A comparison of the reflection indexes characterizing the content of chlorophylls (ChlRI) and anthocyanins (ARI) in leaves showed that the photosynthetic apparatus with a greater potential is generally formed under the influence of fullerene derivatives. Under the influence of these derivatives, the lipid peroxidation intensity also decreased and superoxide dismutase was activated while reactive oxygen species generation in leaves and (or) roots increased (predominantly in barley) or decreased. These changes in plants were the most expressed at fullerenol, C_{60}-threonine and C_{60}-hydroxyproline action. Under stress modeling (UV-B irradiation, 20 kJ/m^2), the UV-resistance of barley plants after not-root treatment with fullerenol, C_{60}-threonine and C_{60}-hydroxyproline, when estimated by the dry weight of the above ground parts and roots, was 10-20 % higher compared to that of the control irradiated plants which were of less weight (by ≈ 33 % for stems and leaves, and by 10-20 % for roots). Thus, the study revealed the positive influence of synthesized amino acid derivatives of fullerene C_{60} and fullerenol on the plant production process and resistance to oxidative stress. High efficiency in small concentrations, low expenses for application and environmental friendliness indicate the perspectiveness of these compounds and necessitate further studying the mechanisms of their action on the soil—plant system to create preparations for use in plant growing.

Keywords: amino acid fullerene C_{60} derivatives, fullerenol, plant production processes, optimization, oxidative stress, resistance, ecologically safe preparations, plant growing

The requirement for biodegradable adaptogens and protectors stimulates the development of innovative forms that provide transportation of macro- and microelements and physiologically active compounds to plants. Prospects in this field are associated with carbon nanomaterials, in particular with water soluble fullerene derivatives [1, 2]. The high lipophilicity of the carbon core of fullerene derivatives ensures their penetration into biomembranes [3-5], the nanoscale size provides a steric conformity to biomolecules, and the π-electrons cloud on the surface allows participation in free radical processes which can be multidirectional depending on the fullerenol concentration, features of the impact object and conditions [3, 6, 7]. Most water-soluble fullerene derivatives are compounds with functionalized hydroxyl, carboxyl and amino groups [1].

Peculiarities and mechanisms of water soluble fullerene derivatives' influence on plants in agro- and ecosystems are poorly studied, since these researches only begin to develop extensively [4, 6, 8, 9]. The water-soluble derivatives are reported to have effects of both inhibition of growth and stimulation of growth, development and productivity. Thus, poly(oxy)hydroxylated fullerene damaged onion cells [10], but promoted the growth of the arabidopsis hypocotyls and green alga *Pseudokirchnerella subcapitata* [6]. Treatment of bitter melon seeds with a solution of poly(oxy)hydroxylated fullerene led to 54 % increase in plant biomass, 128 % increase in yield, and 90 % higher content of useful substances [8]. The positive impact of these compounds on plants is presumably associated with antioxidant activity, such as the ability to bind reactive oxygen species [11-13].

Earlier we reported on the ability of fullerenol C_{60} to prevent oxidative stress in roots of cereals and subapical root thickening after UV-B irradiation of seedlings due to a decrease in the reactive oxygen species (ROS) content. The results of treatment of tested plant seeds (spring barley *Hordeum vulgare* L.) with different concentrations of fullerenol C_{60} revealed its high biological activity [9].

Absorption, translocation and accumulation of C_{60} or C_{70} fullerene derivatives are described in rice, radish, onions, bitter melon, and wheat [8, 10, 14-16]. Water soluble fullerene C_{60} derivatives penetrate through animal and plant cell membranes as lipophilic ions or in neutral form after protonation [4]. On seedlings of wheat (*Triticum aestivum* L.) and radish (*Raphanus sativus* L.) it was shown that the uptake of fullerene derivatives C_{60} and C_{70} by plants depends on fullerene concentrations in the root area and that these compounds are accumulated mainly in the roots [15, 16].

Almost nothing is known about the possible mediated influence on plants of water soluble fullerenes derivatives after they get into the soil. We have shown for the first time that an increase in net productivity and resistance of plants to oxidative stress after the amino acid C_{60} derivatives and fullerenol introduction into the root area or foliage treatment is obviously associated with the established changes in the structure and efficiency of the photosynthetic apparatus and also with the influence on the antioxidant protection system, namely, the intensity of lipid peroxidation, the activity of superoxide dismutase, and the generation of reactive oxygen species.

The aim of the research was to evaluate fullerene C_{60} derivatives impact on the production process and plant resistance to oxidative stress under controlled conditions.

Techniques. Water soluble C_{60} derivatives (fullerenol and C_{60} adducts with L-lysine, L-threonine, L-arginine and L-hydroxyproline amino acids) were obtained by previously developed a one-step synthesis method from individual fullerenes, fullerene mixture or fullerene soot with the use of alkali water solution and an interphase catalyst (TBAH) [17, 18].

The effects of fullerene derivatives on Leningradskaya 6 spring soft wheat (*Triticum aestivum* L.) and Leningradskii and Belogorskii spring barley (*Hordeum vulgare* L.) varieties were studied under controlled favorable conditions and under oxidative stress simulated by UV-B irradiation of the aerial parts of the plants (APHI bio-polygon). Plants were grown in vessels with aerated nutrient solutions, under artificial illumination [19] for 1 month (until appearance of leaves 6-7, stem elongation stage). The light installations equipped with elevating blocks of DHa3-400 lamps (Russia), lighting intensity was 80-90 W/m^2 for photosynthetically active radiation (PAR) with 14 h light period, the air temperature was 25±2 °C at 65±5 % relative humidity. Ten plants were used per vessel, with repeatability of 30 plants per test variant. The nutrient solution in the vessels was aerated permanently, replacement of the solution and pH control were carried out every 3 days. The macro- and microelements composition has been proposed earlier [9].

Synthesized fullerene C_{60} derivatives were introduced into the root area (into aerated nutrient solution) [9] at 1 mg/l concentration or by foliar application (0.1 mg and 15 mg per 1 l of macro- and microelements solution) [9]; the concentrations of the derivatives are based on preliminary research data. The macro- and microelements solution without fullerene derivatives was the control [9]. The pH values of tested solutions were within 6.2-6.9 (the variation of pH in this range does not have a significant effect on plant growth). In variants with foliage application of C_{60} derivatives with macro- and microelement solutions (nanocompositions), plants were sprayed 3 times per vegetation (with 7 days periodicity during the tillering—stem elongation); plants treated with macro- and microelements without C_{60} derivatives were the control.

To create stressful conditions, the plants were exposed to UV-B radiation during stem elongation stage. Before irradiation, the plants were treated 3-fold (7 days periodicity) by foliage application of nanocompositions of fullerene derivatives. Irradiation of the plants started 3 days after the last foliage treatment with nanocompositions. The dose of biologically effective UV-B radiation was 20 kJ/m^2; LE-30 lamps (Russia) with a spectral range of 280-380 nm and maximum intensity at λ = 320 nm were used. Ten plants were used per vessel, with repeatability of 50 plants per variant. Non-irradiated plants treated with macro- and microelements without C_{60} derivatives were the control.

Antioxidant properties of fullerenes were assessed via lipid peroxidation intensity determined by accumulation malonic dialdehyde (MDA), superoxide dismutase activity (SOD) in nitroblue tetrazolium test (by ability to compete for superoxide radical), and generation of reactive oxygen intermediate (ROI) estimated by conversion of adrenaline to adrenochrome which optical density was measured at λ = 480 nm [20-22].

The parameters of leaf reflectance spectra were registered in the range from 400 to 1100 nm with 0.3 nm increments (a fiber-optical spectroradiometric system, Ocean Optics, Inc., USA) at 0.065 nm resolution [23]. The main elements of the system are a spectrometer HR2000, SpectraSuite software, tungsten halogen light source LS-1 (standard), Spectralon standard WS-1, standard probe of reflection/backscattering R-200-7-UV-VIS. Indexes of reflection were calculated to quantitate chlorophylls, flavonoids, anthocyanins in leaf tissue and other photosynthetic parameters.

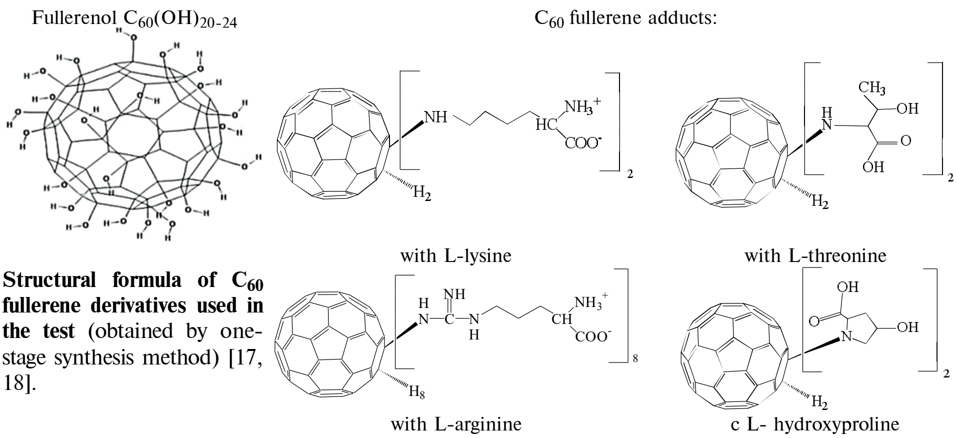
At the end of each greenhouse trial, the biometric parameters of plant growth were measured, including the total weight and biomass of leaves, roots, and stems. Assimilating leaf surface area (S) was calculated by formula:

$$S = (P \times S_1 \times n)/P_1,$$

where S_1 is the area of a cutting; n is the number of cuttings; P is the total leaf weight, g; P_1 is weight of cuttings, g.

Data processing was performed with MS Excel 2003 software and Statistica 8 (StatSoft Inc., USA). The arithmetical mean values for the parameters (*M*) and their confidence range (±SEM) at 95 % probability level by the Student's *t*-test are given. In greenhouse traits, where the sample size was small (up to 30 plants), the significance of the differences between the test variants was also determined by a nonparametric Wilcoxon test.

Results. The figure shows structural formulas of synthesized water soluble C_{60} derivatives, the fullerenol, C_{60} adducts with amino acid L-lysine, L-threonine, L-arginine and L-hydroxyproline. Amino acids for fullerene derivatives synthesis are chosen because lysine, threonine and arginine are indispensable amino acids not synthesized in humans and animals, and hydroxyproline is interesting due to a nonspecific increase in plants stress resistance



Structural formula of C_{60} fullerene derivatives used in the test (obtained by one-stage synthesis method) [17, 18].

Assessment of effects of the fullerene derivatives on spring wheat and spring barley plants indicates that the presence of these compounds in the root area promotes larger leaves area with higher chlorophyll content (Table 1). Thus, in Leningradskaya 6 spring wheat these changes were more pronounced than in Leningradskaya spring barley. The obtained data show that the functional potential of photosynthetic apparatus influenced by fullerene derivatives is higher compared to control, that leads to an increase in the net productivity. Ch. Wang et al. [16] reported about the ability of fullerenol to increase the chlorophyll synthesis in leaves of 7-day old seedlings of spring wheat.

Note, the barley plants treated with amino acid derivatives of fullerene and the corresponding amino acids do not differ significantly in leaf content of chlorophylls (ChlRI) and anthocyanins (ARI) (see Table 1), while in wheat plants the threonine and hydroxyproline derivatives in the nutrient solution have significantly more pronounced stimulating effect on ChlRI and inhibiting effect on ARI than the amino acids themselves.

1. Physiological parameters and net productivity (deviation from control, %) **in Leningradskii spring barley and Leningradskaya 6 spring wheat in the presence of C_{60} fullerene derivatives in the root area** (greenhouse trials)

Compound	Pigment content		LA	Dry weigh			
	ChlRI	ARI		roots	stems	leaves	total
Barley of Leningradskii variety							
$C_{60}(OH)_{20-24}$	+13	–17*	+19*	+105*	+79*	+54*	+67*
C_{60}-arginine	+8	–10	+16*	+38*	+22*	+15*	+27*
Arginine	+9	–15*	+54*	+34*	+22*	+26*	+25*
C_{60}-lysine	+12	–13*	+26*	+28*	+41*	+33*	+32*
Lysine	+13	–19*	+36*	+76*	+67*	+46*	+63*
C_{60}-threonine	+16*	–25*	+15*	+69*	+69*	+32*	+50*
Threonine	+13	–18*	–9	+27*	+7	+2	+10
C_{60}-hydroxyproline	+11	–23*	+0,5	+85*	+60*	+33*	+54*
Hydroxyproline	+8	–13*	+30*	+78*	+30*	+38*	+45*
Wheat of Leningradskaya 6 variety							
$C_{60}(OH)_{20-24}$	+54*	–17*	+89*	+296*	+275*	+177*	+226*
C_{60}-arginine	+23*	–7	+20*	+83*	+174*	+112*	+124*
Arginine	+11	–9	+60*	+77*	+165*	+128*	+124*
C_{60}-lysine	+19*	–10	+60*	+80*	+133*	+107*	+110*
Lysine	+20*	–11	+73*	+96*	+170*	+133*	+134*
C_{60}-threonine	+32*	–24*	+38*	+84*	+188*	+112*	+127*
Threonine	+14*	–8	–37*	+87*	+14*	+3	+20*
C_{60}-hydroxyproline	+47*	–17*	+49*	+138*	+284*	+178*	+197*
Hydroxyproline	+8	–3	–19*	+133*	+40*	+35*	+49*

Note. The averaged data of two tests under controlled conditions (n = 30 in each) are shown. Composition of aerated 20 % nutrient solution with micro- and macronutrients was described earlier [9], day/night is 14/10 h (DNT-400 lamps, 80-90 W/m^2 of photosynthetically active radiation). ChlRI and ARI are reflection indices of the content of chlorophylls (ChlRI) and anthocyanins (ARI) in plant leaves (formulas to calculate the indices were given earlier) [23, 24]; LA is assimilating leaf area. Biomass was weighed at stem elongation phase (leaf 6-7).
* Differences with control are statistically significant at $p < 0.05$.

2. Biomass accumulation in foliar treatment of plants with nanocomposition based on C_{60} fullerene derivatives (greenhouse trial)

Variant	Aerial parts		Roots	
	g, M±SEM	deviation from control, %	g, M±SEM	deviation from control, %
Barley				
Belogorskii variety				
Solution	5.50±0.26		2.73±0.30	
Solution + $C_{60}(OH)_{20-24}$	5.93±0.48	+7.8	2.97±0.31	+8.8
Leningradskii variety				
Solution	2.10±0.20		0.50±0.05	
Solution + $C_{60}(OH)_{20-24}$	1.98±0.20	–5.7	0.46±0.06	–8.0
Solution + C_{60}-threonine	2.00±0.20	–4.8	0.70±0.06*	+40.0*
Solution + threonine	1.97±0.22	–6.2	0.50±0.06	0
Wheat				
Leningradskaya 6 variety				
Solution	0.75±0.14		0.33±0.08	
Solution + $C_{60}(OH)_{20-24}$	0.76±0.18	+1.3	0.31±0.06	–6.1
Solution + C_{60}-threonine	0.79±0.16	+5.3	0.40±0.06*	+21.2*
Solution + threonine	0.75±0.16	0	0.36±0.08	+9.1

Note. In each variant n = 50. Composition of aerated 20 % nutrient solution with micro- and macronutrients was described earlier [9], day/night is 14/10 h (DNT-400 lamps, 80-90 W/m^2 of photosynthetically active radiation). Concentrations of C_{60}-threonine and $C_{60}(OH)_{20-24}$ are 0.1 mg/l and 15 mg/l, respectively. Biomass was weighed at stem elongation phase (leaf 6-7).
* Differences with control are statistically significant at $p < 0.05$.

According to the available data, the induction of anthocyanin synthesis is a nonspecific response to various abiotic stressors, UV-B radiation, soil drought, and nitrogen deficit [23, 24]. It was found that the C_{60} fullerene derivatives introduction into the root area decreased the leaf level of anthocyanins in barley and wheat plants. This indirectly indicates an improvement in the physiological state of plants, which is confirmed by higher growth parameters. Under the effect of C_{60} fullerene derivatives, the roots are extended, plants are higher and have more stems (data not given), which results in higher biomass of leaves, stems, roots and a plant as a whole. While fullerene derivatives are introduced into the root area, the total dry weight (roots + stems + leaves) for both crops overcomes controls by 27-226 % ($p < 0.05$) (see Table 1). In foliar treatment with the nanocomposition of fullerene derivatives, the dry weight of the aerial parts of barley and wheat plants did not differ significantly from the controls, while the root biomass was 8-40 % higher (Table 2).

Parametric tests and nonparametric Wilcoxon test showed the reliability of differences between the control and application of fullerene derivatives. Fullerenol, fullerene C_{60}-oxyproline, and fullerene C_{60}-threonine caused the most pronounced increase in growth indicators compared to control. The nutrient solution combined with hydroxyproline, threonine and, to a lesser extent, arginine derivatives of C_{60} fullerene have significantly higher positive effect on leaves, stems and roots compared to the same solution but containing only a corresponding amino acid. Inversely, in pairwise comparison of amino acid derivatives of fullerenes and the corresponding amino acids, the positive effect of lysine is higher compared to that of lysine adduct of C_{60}.

In our tests, all C_{60}-fullerene derivatives significantly influenced the antioxidant systems of barley and wheat plants, with more apparent effect of the hydroxyproline and threonine derivatives. However, the antioxidant response of these two cereals was somewhat similar, but somewhat different (Table 3). Intensity of LP in leaves and roots decreased by C_{60}-threonine and C_{60}-hydroxyproline, mostly reliably or as a trend. The decrease was more apparent in barley leaves (by 18 and 20 %, $p < 0.05$), in wheat leaves with C60-threonine (by 10 %, $p < 0.05$) and in wheat roots with C_{60}-hydroxyproline (by 30 %, $p < 0.05$). In barley, SOD activity did not significantly change in the leaves and increased by 17-18 % ($p < 0.05$) in the roots under the impact of both amino acid derivatives. Wheat plants manifested a weak tendency to a decrease of this index in leaves with C_{60}-hydroxyproline and C_{60}-threonine and reliable reduction of SOD activity in the roots (by 21 %, $p < 0.05$) with C_{60}-threonine.

3. Antioxidant activity of Leningradsii spring barley plants under root treatment with the C_{60}-hydroxyproline and C_{60}-threonine (greenhouse trial)

Index	Leaves			Roots		
	control value	absolute value	deviation from control, %	control value	absolute value	deviation from control, %
		C_{60}-threonine				
LP, mM/g	0.0065	0.0052*	–20*	0.0069	0.0064	–7
SOD, relative units	1.0680	1.0770	+1	1.0770	1.2635*	+17*
ROS, relative units	3.3300	6.2300*	+87*	0.3300	1.0300*	+212*
		C_{60}-hydroxyproline				
LP, mM/g	0.0065	0.0053*	–18*	0.0069	0.0069	0
SOD, relative units	1.0680	0.9513	–11	1.0770	1.2669*	+18*
ROS, relative units	3.3300	3.8700*	+16*	0.3300	0.6300*	+91*

Note. The averaged data of two tests under controlled conditions (n = 30 in each) are shown. Composition of aerated 20 % nutrient solution with micro- and macronutrients was described earlier [9], day/night is 14/10 h (DNT-400 lamps, 80-90 W/m^2 of photosynthetically active radiation). LP is lipid peroxidation, SOD is superoxide dismutase, ROS is reactive oxygen species.
* Differences with control are statistically significant at $p < 0.05$.

Changes of ROS content in leaves and roots under the effect of amino acid derivatives of C_{60} are the most contrasting in comparing two crops and vary from a reliable increase in barley roots (by 91 and 212 %, $p < 0.05$) and leaves (by 16 and 87 %, $p < 0.05$), and also in wheat roots (by 56 %, $p < 0.05$) with C_{60}-hydroxyproline to a decrease in wheat roots and leaves (by 61 and 71 %, $p < 0.05$) with C_{60}-threonine and a tendency to decline in the wheat leaves with C_{60}-hydroxyproline.

4. Antioxidant activity of Leningradskii spring barley and Leningradskaya 6 spring wheat under foliar treatment with C_{60}-hydroxyproline and C_{60}-threonin (greenhouse trial)

Index	Leaves			Roots		
	control value	absolute value	deviation from control, %	control value	absolute value	deviation from control, %
Barley of Leningradskii variety						
C_{60}-threonine						
LP, mM/g	0.0069	0.0049*	–29*	0.0068	0.0134*	+97*
SOD, relative units	0.7810	1.1925*	+53*	1.3396	1.1148*	–17*
ROS, relative units	5.2000	4.0000*	–23*	0.2700	1.4700*	+444*
C_{60}-hydroxyproline						
LP, mM/g	0.0069	0.0054*	–22*	0.0068	0.0064	–6
SOD, relative units	0.7810	1.2123*	+55*	1.3396	1.0985*	–18*
ROS, relative units	5.2000	5.1000	–2	0.2700	1.5000*	+456*
Wheat of Leningradskaya 6 variety						
C_{60}-threonine						
LP, mM/g	0.0060	0.0057	–5	0.0095	0.0098	+3
SOD, relative units	0.9933	1.1542*	+16*	0.8738	0.9294	+6
ROS, relative units	1.0700	1.9700*	+84*	0.9700	0.0300*	–97*
C_{60}-hydroxyproline						
LP, mM/g	0.0060	0.0046*	–23*	0.0095	0.0067*	–30*
SOD, relative units	0.9933	1.0240	+3	0.8738	0.9360	+7
ROS, relative units	1.0700	0.5000*	–53*	0.9700	0.9700	0

N o t e. The averaged data of two tests under controlled conditions (n = 30 in each) are shown. Composition of aerated 20 % nutrient solution with micro- and macronutrients was described earlier [9], day/night is 14/10 h (DNT-400 lamps, 80-90 W/m^2 of photosynthetically active radiation). LP — lipid peroxidation, SOD — superoxide dismutase, ROS — reactive oxygen species.

* Differences with control are statistically significant at $p < 0.05$.

In foliar treatment of barley and wheat plants with fullerene derivatives, the LP, SOD and ROS values change in a similar way when these compounds enter root area (Table 4). The antioxidant effect of foliar C_{60}-threonine and C_{60}-hydroxyproline was higher in plant leaves, i.e. predominantly, a decrease in LP and ROS content and an increase in SOD activity. In the roots, contrarily, oxidation increased, especially in barley. In barley, foliar treatment with fullerene derivatives, like root treatment, elevated sharply the root level of ROS (by 444 and 456 %, $p < 0.05$), but SOD activity and LP changed in roots oppositely, i.e. SOD significantly decreased and LP increased with C_{60}-threonine and did not change significantly with C_{60}-hydroxyproline. In wheat, foliar C_{60}-hydroxyproline resulted in a significant decrease in LP, while C_{60}-threonine decreased ROS level, with no significant differences from control for other estimated indices.

Such ambiguous results can be due to different sensitivity of the studied varieties to impacts [25]. So, in barley, more responsive to test substances, higher ROS level in roots and leaves upon root treatment, and also in roots under foliar treatment indirectly indicates possible involvement of active oxygen species in constructive plant metabolism, and also, apparently, immunomodulating effect of C_{60}-hydroxyproline and C_{60}-threonine derivatives. This effect is similar to that of a vaccine which activates an immune response to a potentially dangerous factor before its impact with a significant enhancement of plant resistance. Less reactive wheat plants, as compared to barley plants, showed more apparent direct anti oxidative effects of amino acid derivatives, both upon root and foliar treatment.

Thence, the influence of studied fullerene derivatives on growth and net productivity of plants is associated with their significant regulatory impact on synthesis of plant pigments, efficiency of photosynthetic apparatus, and also on antioxidant protection against oxidative stress.

Earlier we revealed the ability of fullerenol, a polyoxyhydroxylated fullerene derivative, to prevent oxidative stress in roots of cereal seedlings via a decline of free radical level under stressors (UV-B radiation, salinity or salicylate in the root area) and, thus, to enhance plant resistance during early phases of growth [9].

Detected effects of fullerene derivatives on barley and wheat plants under favorable conditions gave us reason to predict an increase in plant resistance to factors that cause oxidative stress. Our experiments to simulate stress during stem elongation which is the most susceptible period of cereal plant growth, confirm protective functions of fullerene derivatives upon foliar application. By the example of Leningradskii spring barley, it was found that on day 2 after UV-B irradiation the chlorophyll content decreased significantly (by 4 %) in the leaves of control plants and did not change in the leaves of plants pretreated with C_{60}-threonine (see Tables 2, 5). The same was observed on day 7 after UV-B radiation. The leaf level of anthocyanins on day 2 after irradiation increased by 20 % in control plants, by 13 % in those treated with C_{60}-threonine and by 8 % after treatment with threonine. After 7 days, the anthocyanins in the control irradiated plants remained 20 % higher than in control (without irradiation), while this level increased by 17 % with C_{60}-threonine and by 30 % with threonine. This suggests that the impact UV-B radiation stress on plants pretreated with C_{60}-threonine is less significant compared to that of control irradiated plants and the plants pretreated with threonine.

5. Leaf pigment contents in Leningradskii barley variety under foliar treatment with amino acid derivatives of fullerene (C_{60}-threonine as the example) (greenhouse trial)

Variant	ChlRI, relative units		ARI, relative units		ChlRI, % to control		ARI, % to control	
	day 2	day 7	day 2	day 7	day 2	day 7	day 2	day 7
Solution	0.512	0.478	0.521	0.482	100	100	100	100
Solution + C_{60}-threonine	0.523	0.484	0.486	0.494	102	101	93	103
Solution + threonine	0.508	0.497	0.479	0.506	99	104	92	105
Solution + UV-B	0.492	0.456	0.627*	0.578*	96	95	120*	120*
Solution + UV-B + C_{60}-threonine	0.512	0.494	0.587*	0.565*	100	103	113*	117*
Solution + threonine + UV-B	0.507	0.471	0.563*	0.624*	99	99	108*	130*

N o t e. In each variant *n* = 50. Composition of aerated 20 % nutrient solution with micro- and macronutrients was described earlier [9], day/night is 14/10 h (DNT-400 lamps, 80-90 W/m^2 of photosynthetically active radiation). C_{60}-threonine concentration is 0,1 mg/l. UV is UV-B irradiation of plants at 20 kJ/m^2; plants not treated with C_{60}-threonine and not subjected to UV-B irradiation are the control.
* Differences with control are statistically significant at $p < 0.05$.

6. Spring barley plant biomass after UV-B irradiation (deviation form control, %) under foliar application of nanocomposition based on C_{60}-fullerene derivatives (greenhouse trial)

Variant	Aerial parts	Roots
	Belogorskii variety	
Solution + $C_{60}(OH)_{20\text{-}24}$	+7.8	+8.8
Solution +UV-B	–32.7*	–9.5
Solution + UV-B + $C_{60}(OH)_{20\text{-}24}$	–13.1	+11.7
	Leningradskii variety	
Solution + C_{60}-threonine	–4.8	+40.0*
Solution + threonine	–6.2	0
Solution+ UV-B	–33.3*	–20.0*
Solution+ UV-B + C_{60}-threonine	–23.8*	–2.0
Solution + threonine + UV-B	–28.6*	–8.0

N o t e. In each variant *n* = 50. Composition of aerated 20 % nutrient solution with micro- and macronutrients was described earlier [9], day/night is 14/10 h (DNT-400 lamps, 80-90 W/m^2 of photosynthetically active radiation). Concentration of C_{60}-threonine is 0.1 mg/l, of $C_{60}(OH)_{20\text{-}24}$ — 15 mg/l. Biomass was weighed at stem elongation phase (leaf 6-7). UV-B dose is 20 kJ/m^2.

* Differences with control are statistically significant at $p < 0.05$.

As per weight of the aerial parts and roots, the barley plant tolerance to UV-B irradiation was significantly higher (by 10-20 %) after treatment with nanocomposition. In the control irradiated plants under impact of the stressor, the weight of the aerial parts reduced by approximately 33 %, of roots — by 10-20 % (Table 6). Note a tendency to more pronounced effect of a threonine derivative of fullerene compared to threonine.

Thus, C_{60} fullerene derivatives, the fullerenol and C_{60} fullerene adducts with threonine, lysine, arginine, hydroxyproline, stimulate growth and net productivity in spring wheat and barley under controlled conditions. This is due to the regulatory effect on synthesis of photosynthetic pigments and activity of photosynthetic apparatus, as well as to the antioxidant effect that enhances the protection of plants from oxidative stress. These changes are the most apparent under application of fullerenol, C_{60}-threonine and C_{60}-hydroxyproline. The stress resistance under UV-B radiation of barley plants treated with these compounds was 10-20 % higher than in the irradiated control plants. The obtained data indicate the necessity for further study of the impact of water soluble fullerene derivatives on plants and their habitat to create effective and environmentally friendly preparations for crop production in which high efficiency at low concentrations are combined with low costs due to a solid powder form (unlike liquid analogues).

REFERENCES

1. Piotrovskii L.B., Kiselev O.I. *Fullereny v biologii* [Fullerenes in biology]. St. Petersburg, 2006 (in Russ.).
2. Semenov K.N., Charykov N.A., Keskinov V.A. Fullerenol synthesis and identification. Rroperties of fullerenol water solutions. *J. Chem. Eng. Data*, 2011, 56: 230-239 (doi: 10.1021/je100755v).
3. Piotrovskii L.B. *Rossiiskie nanotekhnologii*, 2007, 2(7-8): 6-18 (in Russ.).
4. Andreev I., Petrukhina A., Garmanova A., Babakhin A., Andreev S., Romanova V., Troshin P., Troshina O., DuBuske L. Penetration of fullerene C_{60} derivatives through biological membranes. *Fullerenes, Nanotubes, and Carbon Nanostructures*, 2008, 16: 89-102 (doi: 10.1080/15363830701885831).
5. Kornev A.B., Troshina O.A., Troshin P.A. Biologicheski aktivnye proizvodnye fullerenov, metody ikh polucheniya i primenenie v meditsine. V knige: *Organicheskie i gibridnye nanomaterialy: tendentsii i perspektivy* /Pod redaktsiei V.F. Razumova, M.V. Klyueva [Organic and hybrid nanomaterials: trends and prospects. V.F. Razumov, M.V. Klyuev (eds.)]. Ivanovo, 2013: 392-485 (in Russ.).
6. Gao J., Wang Y., Folta K.M., Krishna V., Bai W., Indeglia P., Georgieva A., Nakamura H., Koopman B., Moudgi B. Polyhydroxy fullerenes (fullerols or fullerenols): beneficial effects on growth and lifespan in diverse biological models. *PLoS ONE*, 2011, 6(5): e19976 (doi: 10.1371/journal.pone.0019976).
7. Zhao Y.Y., Yang B., Tang M.L. Guo Q-Ch., Chen Ju-T., Wen L-P., Wang M. Concentration-dependent effects of fullerenol on cultured hippocampal neuron viability. *Int. J. Nanomed.*, 2012, 7: 3099-3109 (doi: 10.2147/IJN.S30934).
8. Kole C., Kole P., Randunu K.M., Choudhary P., Podila R., Ke P.C., Rao A.M., Marcus R.K. Nanobiotechnology can boost crop production and quality: first evidence from increased plant biomass, fruit yield and phytomedicine content in bitter melon (*Momordica charantia*). *BMC Biotechnol.*, 2013, 13: 37-58 (doi: 10.1186/1472-6750-13-37).
9. Panova G.G., Ktitorova I.N., Skobeleva O.V., Sinjavina N.G., Charykov N.A., Semenov K.N. Impact of polyhydroxy fullerene (fullerol or fullerenol) on growth and biophysical characteristics of barley seedlings in favourable and stressful conditions. *Plant Growth Regul.*, 2016, 79: 309-317 (doi: 10.1007/s10725-015-0135-x).
10. Chen R., Ratnikova T.A., Stone M.B., Lin S., Lard M., Huang G., Hudson J.S., Ke P. C. Differential uptake of carbon nanoparticles by plant and Mammalian cells. *Small*, 2010, 6: 612-617 (doi: 10.1002/smll.200901911).
11. Dugan L.L., Lovett E.G., Quick K.L., Lotharius J., Lin T.T., O'Malley K.L. Fullerene-based antioxidants and neurodegenerative disorders. *Parkinsonism Relat. Disord.*, 2001, 7: 243-246 (doi: 10.1016/S1353-8020(00)00064-X).
12. Gharbi N., Pressac M., Hadchouel M., Szwarc H., Wilson S.R., Moussa F. [60]Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett.*, 2005, 5: 2578-2585 (doi: 10.1021/nl051866b).
13. Yin J.J., Lao F., Fu P.P., Wamer W.G., Zhao Y., Wang P.C., Qiu Y., Sun B., Xing G., Dong J., Liang X.-J., Chen C. The scavenging of reactive oxygen species and the potential for cell protection by functionalized fullerene materials. *Biomaterials*, 2009, 30(4): 611-621 (doi: 10.1016/j.biomaterials.2008.09.061).
14. Lin S., Reppert J., Hu Q., Hudson J.S., Reid M.L., Ratnikova T.A., Rao A.M., Luo H., Ke P.C. Uptake, translocation, and transmission of carbon nanomaterials in rice plants. *Small*, 2009, 5: 1128-1132 (doi: 10.1002/smll.200801556).
15. Avanasi R., Jackson W.A., Sherwin B., Mudge J.F., Anderson T.A. C60 fullerene soil sorption, biodegradation, and plant uptake. *Environ. Sci. Technol.*, 2014, 48(5): 2792-2797 (doi: 10.1021/es405306w).
16. Wang C., Zhang H., Ruan L., Chen L., Li H., Chang X.-L., Zhang X., Yang S.-T. Bioaccu-

mulation of ^{13}C-fullerenol nanomaterials in wheat. *Environ. Sci.: Nano*, 2016, 4: 1-7 (doi: 10.1039/C5EN00276A).
17. Semenov K.N., Charykov N.A., Namazbaev V.I., Keskinov V.A. *Sposob polucheniya smesi fullerenolov*. Patent RF na izobretenie RU 2495821 C2. ZAO ILIP (RU), OOO Agrofizprodukt (RU). № 2010122963/05. Zayavl. 04.06.2010. Opubl. 20.10.2013. Byul. № 29 [Method for the preparation of a mixture of fullerenols. Patent RU 2495821 C2. ZAO ILIP (RU), OOO Agrophysprodukt (RU). № 2010122963/05. Appl. 04.06.2010. Publ. 20.10.2013. Bul. № 29 (in Russ.).
18. Semenov K.N., Charykov N.A., Keskinov V.A., Keskinova M.V., Saf'yannikov N.M., Shubina V.A. *Sposob polucheniya fullerenolov*. Patent RF na izobretenie RU 2481267 C2. OOO «Biogel'tek» (RU). № 2011106276/05. Zayavl. 11.02.2011. Opubl. 10.05.2013. Byul. № 13 [Method for the preparation of fullerenols. Patent RU 2481267 C2. OOO «Biogeltek» (RU). № 2011106276/05. Appl. 02/11/2011. Publ. . 10.05.2013. Bul. № 13] (in Russ.).
19. Panova G.G., Chernousov I.N., Udalova O.R., Aleksandrov A.V., Karmanov I.V., Anikina L.M., Sudakov V.L. *Doklady RASKHN*, 2015, 4: 17-21 (in Russ.).
20. Purvid A.C., Shewfeld R.L., Gegogeine J.W. Superoxide production in mitochondria isolated from green bell pepper fruit. *Physiologia Plantarum*, 1995, 94: 743-749 (doi: 10.1111/j.1399-3054.1995.tb00993.x).
21. Lukatkin A.S. *Fiziologiya rastenii*, 2002, 49: 697-702 (in Russ.).
22. Nekrasova G.F., Kiseleva I.S. *Ekologicheskaya fiziologiya rastenii* [Plant ecophysiology]. Ekaterinburg, 2008 (in Russ.).
23. Yakushev V.P., Kanash E.V., Osipov Yu.A., Yakushev V.V., Lekomtsev P.V., Voropaev V.V. [Optical criteria during contact and distant measurements sowing state of wheat and photosynthesis effectiveness on the background of deficit of mineral nutrition]. *Agricultural Biology*, 2010, 3: 94-101 (in Russ.).
24. Kanash E.V., Panova G.G., Blokhina S.Yu. Optical criteria for assessment of efficiency and adaptogenic characteristics of biologically active preparations. *Acta Horticulturae*, 2013, 1009: 37-44 (doi: 10.17660/ActaHortic.2013.1009.2).
25. Kanash E.V., Ermakov E.I. V sbornike: *Reguliruemaya agroekosistema v rastenievodstve i ekofiziologii* [Control of agroecosystems in plant growing and ecophysiology]. St. Petersburg, 2007: 232-253 (in Russ.).