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SOIL CHEMISTRY

The Effect of Humic Acids and Their Complexes with Iron on the Functional Status of Plants Grown under Iron Deficiency

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Abstract—The effect of humic acids (HAs) and their iron complexes (Fe–HAs) on the input of the main mineral elements into wheat seedlings, as well as on the efficiency of photosynthesis and the lipid profile of plants, under iron deficiency has been studied. The input of iron from Fe–HA complexes and its predominant accumulation in roots are demonstrated. It is found that HAs increase the efficiency of photosynthesis due to enhanced electron transport in photosystem II. It is shown that the application of HAs and Fe–HAs is accompanied by an enhanced input of Zn into plants, which could increase the antioxidant status of plants under iron deficiency conditions. In addition, a pronounced increase in the content of lipids in plants is revealed, which is indicative of the effect of HAs on plant metabolism. The obtained results suggest that the positive effect of Fe–HAs and HAs on plants under iron deficiency conditions is due to a combination of factors, among which the effect of HAs on the antioxidant status of plants and the plant lipid metabolism predominates.

Keywords: physiological activity of humic acids, mineral nutrition of plants, iron deficiency, lipid profile, chlorophyll fluorescence

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INTRODUCTION

Iron fulfils a number of functions in plants, the most important of which are the participation in the synthesis of chlorophyll and auxins (iron enters in enzymes catalyzing these processes) and the transfer of electrons in different redox processes (iron enters in cytochromes and ferredoxins). This makes iron a key element for energetic processes in plants: photosynthesis, respiration, nitrogen assimilation, and synthesis of plant enzymes [35]. The insufficient input of iron into plants disturbs photosynthesis and, hence, reduces the total productivity. This is primarily due to the decrease in the contents of chlorophyll and carotenoids and the inhibition of electron transport in chloroplasts. In addition, an increased formation of active oxygen under iron deficiency is observed in chloroplasts, including the formation of superoxide anion radical $O_2^{\bullet-}$ due to the activation of the Mehler reaction, i.e., the transport of electrons from water to oxygen at the manganese cluster of the oxygen-releasing complex (ORC). Therefore, iron-deficient plants need to increase the protection for membrane structures of chloroplasts, primarily their lipid components [5].

In the soil, the content of iron is higher than that of any other microelement; it is usually no less than 2–3% even in meager soils. However, among the elements essential for the vital activity of plants, iron is the most dependent on changes in the acidity and redox potential of the environment [2]. Favorable conditions for the reduction of Fe^{3+} to Fe^{2+} are created in acid soils, especially hydromorphic ones, which favor an increase in the solubility of iron compounds. Therefore, plants in acid soils can be inhibited by iron salts: chloride or sulfate occurring in soil solution [4]. In neutral and slightly alkaline soils, on the contrary, the mobility of iron decreases abruptly, and many plants, including fruit, berry, and vegetable crops suffer from its deficiency. In the pH range between 7 and 9, soluble mineral iron mainly occurs in the soil in the forms of $Fe(OH)_2^+$, $Fe(OH)_3$, and $Fe(OH)_4^-$. Their concentrations in the solution are about 10^{-10} M, while the concentration of soluble iron necessary for the normal growth of plants should be higher by sev-

eral orders of magnitude: 10^{-6}–10^{-5} M [1]. Hence, iron in the soil with high pH values and good aeration conditions mainly occurs as Fe^{3+} and is hardly available to plants. Therefore, its complexes with soil organic matter, especially humic acids (HAs), represent an important reserve of plant-available iron.

It is commonly accepted that HAs favor the growth and development of plants by facilitating the input of different meso- and microelements, including iron, into them [34]. This effect is most manifested under deficiency of some nutrient [34]. Therefore, it is believed that iron chlorosis can be corrected using HAs, in the presence of which the input of iron into plants is facilitated [14, 28]. The ability of HAs to facilitate the input of iron into plants is usually explained by their chelating activity for iron, which ensures the presence of plant-available iron in the environment [10].

However, the impact of HAs themselves on plants under these conditions is insufficiently understood. Isolated publications [6] indicate the possibility of adsorption and efficient utilization of many ash elements by plants not only in their mineral form, but also in the form of mineral–humus compounds. Using Fe-labeled humin-like substances, Fokin [6] demonstrated the input of such complexes into plants and suggested the presence of a special mechanism for the selective uptake of complexes saturated with mineral nutrients by plants from solutions.

Using tritium-labeled HAs, we showed earlier that natural HAs also can come into living organisms, including bacteria [24], fungi [19], and plants [23]. Thus, the observed positive effect of HAs under iron deficiency conditions can be due not only to their ability to facilitate the input of iron into plants, but also to the physiological activity of HAs entered into living organisms.

The aim of this work was to assess the input of iron into plants in the form of complexes with HAs and their effect on the functional status of plants under iron chlorosis. The functional status of plants was assessed by the thorough analysis of photosynthesis (one of the main energetic processes in plants) and the study of the lipid profile (the main component in membrane construction).

OBJECTS AND METHODS

An HA preparation from leonardite was used in experiments. The preparation was isolated and purified from ash elements according to the International Humic Substances Society procedure [13]. The elemental composition of HAs determined on a Leco CHN 2000 elemental analyzer was as follows (dry ash-free basis), %: C, 59.9; H, 2.4; N, 1.3; O, 36.8. The content of iron in HAs was determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) after wet incineration according to the reported procedure [8] on an Intrepid II XLD (Thermo Fisher Scientific, USA). The content of Fe in HAs was 0.04%.

Complexes of iron with HAs (Fe–HAs) were synthesized according to the earlier developed procedure [15]. For this purpose, 10 mL of a solution of Mohr's salt $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ containing 20 g Fe/L was added by drops to 200 mL of a 10 g/L HA solution, pH 9.0 being maintained with a 0.5 M NaOH solution. The obtained solution was shaken for 12 h and then centrifuged for 30 min at 5000 g; the precipitate was discarded. Fe–HA complexes were synthesized immediately before utilization. The content of Fe–HA iron, determined by ICP-AES as described above, was 7.34%.

To grow soft wheat (*Triticum aestivum* L.), which was used as a test plant, its seeds were preliminarily germinated at 24°C in a thermostat for 10 days. Then, wheat seedlings were transferred to polyethylene containers of 15 L (150 plants/container) containing the complete Knop nutrient medium and put in a growth chamber (photoperiod 18/6; illumination 200 lx) for further growth. The nutrient medium composition was as follows (g/L): KNO_3, 2.02; $Ca(NO_3)_2 \cdot 4H_2O$, 0.12; $Mg(NO_3)_2 \cdot 6H_2O$, 0.26; K_2SO_4, 0.17; KH_2PO_4, 0.14; $CaSO_4 \cdot 2H_2O$, 0.086 (added as a powder); $Na_2B_4O_7 \cdot 10H_2O$, 1.14×10^{-3}; $NaMoO_4 \cdot 2H_2O$, 1.02×10^{-3}; Cu-EDTA, 0.38×10^{-3}; Mn-EDTA, 7.60×10^{-3}; Zn-EDTA, 0.75×10^{-3}. Fe-EDTA at a concentration of 38.2×10^{-3} g/L was also added to the medium for growing control plants. Ten days later, the plants were transferred to the test solutions (Table 1). In the iron-containing test solutions, the concentration of iron corresponded to its concentration in the Knop solution. The content of HAs was 98 mg/L. The biomass of plants was determined 5 days after the transfer of plants to the test solutions. The experiments were performed in triplicate.

To assess the input rate of the main mineral elements, the contents of Ca, Fe, Mg, Mn, P, S, B, Cu, Si, and Zn in the test solutions were determined by ICP-AES 24 h after the beginning of the experiment. The accumulation of these elements in plant tissues was estimated from the analysis of their contents in roots and shoots at the end of the experiment (after 5 days). The preliminary wet incineration of plant samples was successively performed with hot nitric and chloric acids according to the described procedure [8]. The contents of mineral elements in incinerates were analyzed by ICP-AES on an ICP-OES 720-ES spectrometer (Agilent Technologies, USA).

To assess the changes in the lipid profile of plants 24 h after the beginning of the experiment, three plants were taken from each treatment, and lipids were isolated with hot isopropanol using the procedure described in [27] modified according to [21]. Individual lipid classes in the obtained extracts were analyzed

Table 1. Experimental design and brief description of the treatments

Treatment	Growth medium	Test solution
Control	Knop solution	Knop solution
–Fe	Fe-free Knop solution	Fe-free Knop solution
Fe–EDTA	Fe-free Knop solution	Knop solution with Fe–EDTA
HAs	Fe-free Knop solution	Fe-free Knop solution with HAs
Fe–HAs	Fe-free Knop solution	Knop solution with Fe–HAs

by two-dimensional thin-layer chromatography on silica gel 60 plates (10 × 10 cm; Merck, Germany). The analysis of polar lipids included chromatography using a chloroform–methanol–water mixture (65 : 25 : 4) as a mobile phase in the first direction and a chloroform–acetone–methanol–acetic acid–water mixture (50 : 20 : 10 : 10 : 5) in the second direction. The separation of neutral lipids was performed using a toluene–hexane–formic acid mixture (70 : 30 : 0.5) and a hexane–diethyl ether–formic acid mixture (30 : 20 : 0.5) over a path corresponding to two-thirds of the plate height in the same direction. The content of lipids on the plates was determined on a Denskan densitometer (Lenkhrom, Russia) after visualization by heating the plates in a 5% solution of H_2SO_4 in methanol.

To assess the functional status of plants, the main parameters characterizing the photosynthetic activity of plants were measured 5 days after the beginning of the experiment. The measurements were performed using a Dualex 4 multifunctional fluorometer (Force-A, France). The following parameters were registered: chlorophyll content, electron transport rate (ETR) from photosystem II (FSII), nitrogen balance index (NBI), and flavonoid content in leaf epidermis.

The results of experiments were presented as the mean value ± standard deviation. The significance of differences was estimated using the *t*-test at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The performed experiments showed that the growing of wheat plants on an iron-free nutritive solution (treatment –Fe) appreciably reduced the shoot biomass but had no effect on the root biomass (Fig. 1). This fact well agrees with numerous proofs for the inhibition of plant development under iron deficiency [35].

The application of HAs or iron-containing preparations resulted in no significant changes in plant biomass, which could be related to the short duration of the experiment. Nonetheless, the studied preparations affected the main parameters characterizing the efficiency of photosynthesis in plants (Fig. 2). A decrease in the content of chlorophyll from 35 to 21 mg/cm^2 was observed under iron deficiency (Fig. 2a), while the transfer of plants to nutritive mediums containing iron in the form of its complexes with EDTA and HAs increased the content of chlorophyll to 27 and 29 mg/cm^2, respectively; these values did not differ

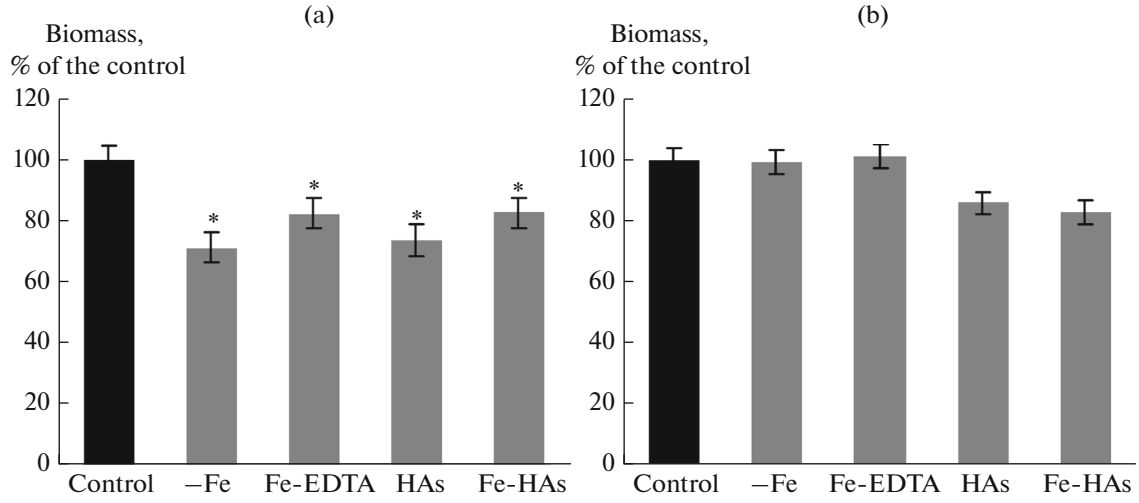

Fig. 1. Effect of iron-containing preparations and HAs on the accumulation of (a) shoot and (b) root biomass by plants; here and below, the values significantly different from the control ($P < 0.05$) are denoted by asterisks.

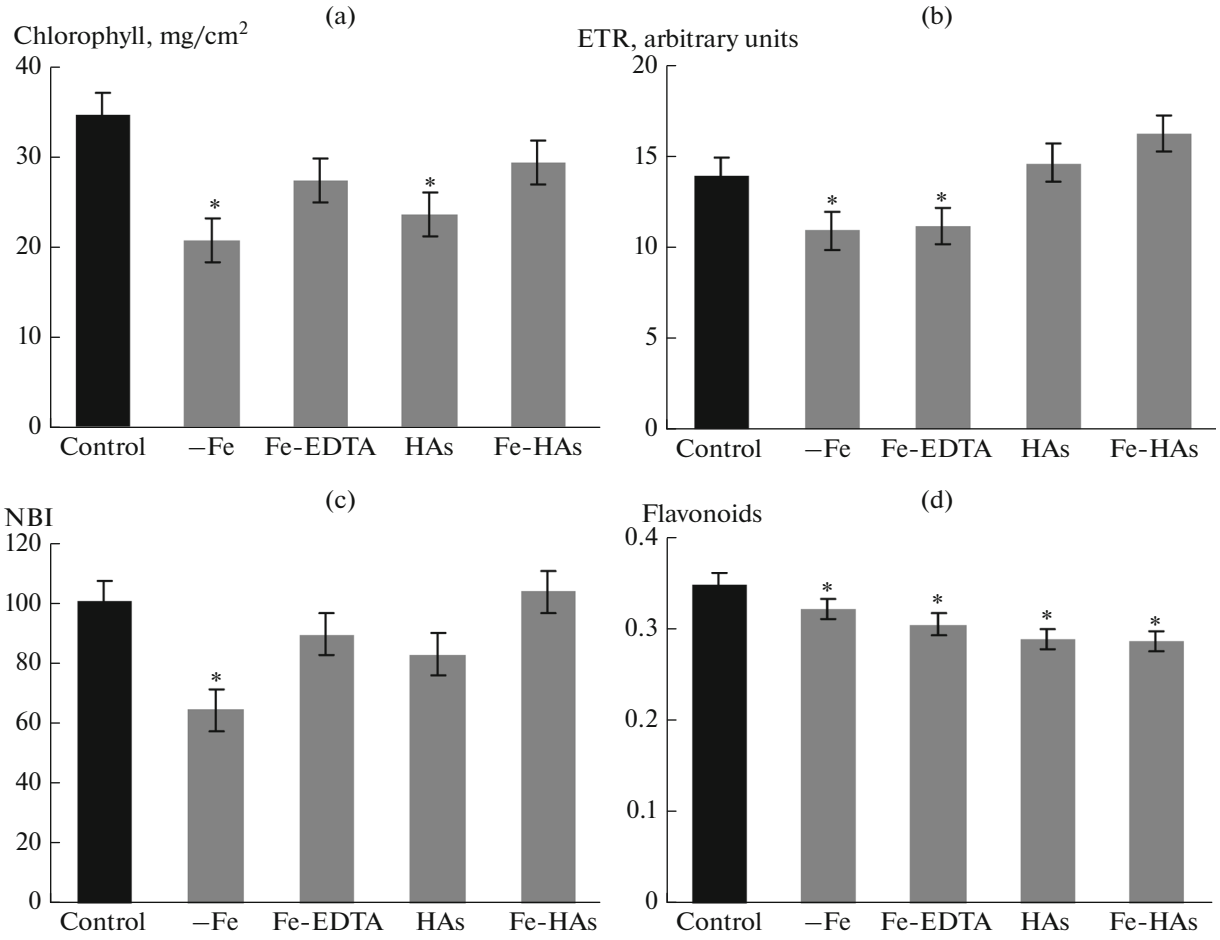

Fig. 2. Effect of iron-containing preparations and HAs on (a) chlorophyll content in plants, (b) electron transport rate ETR, (c) nitrogen balance index NBI, and (d) flavonoid content.

significantly from the control values at the selected significance level. The addition of HAs had no effect on the content of chlorophyll, which could be related to the conservation of iron deficiency conditions in this case.

Although no increase in the content of chlorophyll was noted in the presence of HAs, the both HA-containing preparations (HAs and Fe–HAs) positively affected the ETR and increased it to the control level (Fig. 2b). It should be noted that the complex of Fe with EDTA had no effect on this parameter. This indicates that the enhancement of electron transport in this case is due to the effect of the Fe–HA humic component rather than that of iron.

The studied preparations did not differ from each other in effect on nitrogen balance (Fig. 2c). Under iron deficiency, the NBI value decreased from 101 to 65, which agreed with the available data on the decrease in the content of protein under these conditions [35]. After the addition of Fe–EDTA, HAs, and Fe–HA complexes, the NBI value increased to 84–106 and did not differ significantly from the control values. The revealed positive effect of the preparations on the NBI indicates that the treatment of plants with both iron-containing preparations and HAs under iron chlorosis conditions enhances primary metabolism. It is believed that the optimum growth conditions favor the activation of primary metabolism and the synthesis of proteins, which results in an increase in the NBI value. Under nonoptimum growth conditions, on the contrary, an enhancement of secondary metabolism is observed, which is manifested in the biosynthesis of flavonoids by plants; therefore, their content can serve as an indicator of the stressed state of plants [12], including iron chlorosis [35]. However, the performed experiments showed that the estimation of the functional status of plants from the content of flavonoids by the selected method was insufficiently informative under iron deficiency (Fig. 2d). Iron deficiency resulted in a significant decrease of this parameter rather than in its increase. The observed effect can be related to methodological problems arising at the determination of flavonoid content from the decrease of chlorophyll fluorescence in the leaf mesophyll because of the shielding effect of flavonoids in epider-

Table 2. Main nutrients in test solutions 24 h after the beginning of the experiment

Element		Control	–Fe	Fe–EDTA	HAs	Fe–HAs
Ca	mg/L	58 ± 2	47 ± 2*	40 ± 2*	41 ± 2*	39 ± 2*
Fe		6.5 ± 0.1	0.1 ± 0.1*	6.3 ± 0.1*	0.3 ± 0.1*	5.0 ± 0.1*
Mg		31 ± 1	29 ± 1	30 ± 1	25 ± 1*	25 ± 1*
Mn		1.10 ± 0.04	1.02 ± 0.04*	1.04 ± 0.04	0.91 ± 0.03*	0.88 ± 0.03*
P		45 ± 2	40 ± 2*	43 ± 2	34 ± 1*	34 ± 1*
S		69 ± 3	58 ± 2*	54 ± 2*	50 ± 2*	55 ± 2*
B	μg/L	197 ± 4	169 ± 3*	188 ± 4*	142 ± 3*	141 ± 3*
Cu		74 ± 3	71 ± 3	72 ± 3	62 ± 2*	61 ± 2*
Si		39 ± 5	28 ± 6*	35 ± 5	138 ± 9*	123 ± 10*
Zn		167 ± 3	146 ± 3*	146 ± 3*	118 ± 2*	117 ± 2*

* Significantly differs from the control ($P < 0.05$).

mis, which results in a decrease in the amount of light arriving to the chlorophyll. When a Dualex fluorometer is used for estimating the content of flavonoids, the intensity ratio of chlorophyll fluorescence at the excitation in the ultraviolet region (375 nm), where a part of the light is absorbed by flavonoids, to that in the visible region (650 nm), where there is no light absorption by flavonoids, is calculated [16]. Thus, the content of flavonoids determined on a Dualex fluorometer directly depends on their extinction coefficient at 375 nm. It is believed that the main flavonoids in wheat epidermis are saponarin and lutonarin, which have different absorption spectra [35]. Consequently, the changes in the contents of these flavonoids, which are observed in the stressed state of plants [20], can affect the total absorption of light at 375 nm. Therefore, the variation in the content of flavonoids determined by the comparison of chlorophyll fluorescence values in the ultraviolet and visible spectral regions could be due to different proportions of the main flavonoids in leaf epidermis rather than to the changes in the contents of flavonoids. It should be noted that the comparison of plants pregrown under similar conditions (without available iron) showed that the addition of each of the studied preparations reduced the content of flavonoids (Fig. 2d). The effect was most manifested for HA preparations. The antioxidant function is one of the main functions of flavonoids; therefore, it may be suggested that the lowest content of free radicals in plants was in the presence of HAs.

Thus, the deficiency of iron in the nutrient medium expectedly resulted in a decrease in plant biomass, chlorophyll content, and ETR and NBI values. The positive effect of the studied preparations depended on the presence of iron and HAs in them. The addition of iron-containing preparations positively affected the synthesis of chlorophyll, while the presence of HAs increased the ETR. To reveal the reasons for the observed differences, the effect of the studied preparations on the uptake of iron and some other nutrients by plants was then studied. It was found that changes in the contents of major nutrients in the studied solutions are observed already 24 h after the addition of preparations (Table 2).

In all treatments with the pregrowth of plants in the absence of iron, lower concentrations of almost all elements were observed than in the control treatment. An exception was provided by silicon, whose concentration increased at the addition of HA-containing preparations. This could be related to the insufficient purification of the HA preparation from silicon in this case.

The changes in the uptake of elements under iron deficiency in the nutritive solution can be due to the misbalance of mineral nutrition inducing the synthesis of different low-specific iron transporters in plants. For example, iron-regulated transporter 1, whose biosynthesis is enhanced under iron chlorosis, also participates in the uptake of Zn, Mn, and Cd by plants [17]. In addition, phytosiderophores released by plants of the family Poaceae under iron deficiency are also not specific iron-chelating agents; they can form complexes with other metals [9]. This supposition is confirmed by the lower concentrations of Zn and Mn in solutions of the treatment without iron addition.

The concentration of iron in the solutions with the added iron-containing preparations Fe–EDTA and Fe–HA decreased 24 h after the beginning of the experiment from 6.5 to 6.3 and 5.0 mg/L, respectively. This indicates a more rapid disappearance of iron from the solution in the case of the Fe–HA complex. An analysis of iron content in wheat roots and shoots (Table 3) confirmed that the maximum content of iron in plants, which exceeded not only the values in the control, but also those in the treatment with the addition of Fe–EDTA, was revealed at the addition of iron in the form of its HA complex. Nonetheless, a high content of iron at the addition of Fe–HA was noted only in plant roots (1032 mg/kg), while its content in the aboveground part of plants was relatively low

Table 3. Effect of iron-containing preparations and HAs on the contents of iron and some other nutrients in wheat plants

Element		Control	–Fe	Fe–EDTA	HAs	Fe–HAs
		Roots				
Ca	g/kg	8.0 ± 0.3	10.9 ± 0.4*	9.4 ± 0.4*	5.8 ± 0.2*	10.1 ± 0.4*
Mg	g/kg	1.7 ± 0.2	1.7 ± 0.1	1.4 ± 0.2	2.9 ± 0.2*	1.4 ± 0.2
P	g/kg	6.8 ± 0.3	9.3 ± 0.4*	7.5 ± 0.3*	6.7 ± 0.3	8.6 ± 0.3*
S	g/kg	2.9 ± 0.1	3.9 ± 0.2*	3.2 ± 0.1	1.8 ± 0.1*	2.8 ± 0.2
Fe	mg/kg	613 ± 25	93 ± 4*	713 ± 29*	345 ± 14*	1032 ± 35*
Cu	mg/kg	5 ± 1	6 ± 1	6 ± 1	6 ± 1	7 ± 2
Mn	mg/kg	6 ± 1	5 ± 1	6 ± 1	16 ± 2*	7 ± 1*
Si	mg/kg	26 ± 1	33 ± 3*	27 ± 3	14 ± 1*	34 ± 3*
Zn	mg/kg	18 ± 1	21 ± 1*	22 ± 2*	27 ± 3*	26 ± 4*
		Shoots				
Ca	g/kg	12.3 ± 0.5	12.1 ± 0.5	13.3 ± 0.5	4.8 ± 0.2*	11.3 ± 0.6
Mg	g/kg	2.5 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.3 ± 0.1
P	g/kg	8.4 ± 0.3	7.0 ± 0.3*	7.9 ± 0.3	7.5 ± 0.2*	6.6 ± 0.2*
S	g/kg	4.5 ± 0.2	4.7 ± 0.2	4.8 ± 0.2	2.0 ± 0.1*	4.5 ± 0.2
Fe	mg/kg	54 ± 2	37 ± 1*	98 ± 2*	37 ± 1*	44 ± 1*
Cu	mg/kg	24 ± 3	10 ± 2*	7 ± 2*	6 ± 2*	9 ± 2*
Mn	mg/kg	9 ± 3	11 ± 1	15 ± 3	24 ± 4*	10 ± 2
Si	mg/kg	86 ± 3	38 ± 4*	32 ± 3	56 ± 4*	35 ± 3*
Zn	mg/kg	37 ± 3	24 ± 2*	22 ± 2*	37 ± 3	38 ± 3

* Significantly differs from the control ($P < 0.05$).

(44 mg/kg) and did not reach the values in the control (54 mg/kg) or in the treatment with the addition of Fe–EDTA (98 mg/kg). This indicates the accumulation of iron in roots, which can be due to the predominant adsorption of Fe–HA on the surface of roots and the limited input of adsorbed iron into the vascular system of plants.

The deficiency of iron in the nutritive solution affected the input of several elements (P, Mn, Si, and Zn), which confirms the importance of iron for plant metabolism, as was repeatedly demonstrated earlier [32, 33]. An analysis of the effect of the studied preparations on the input of these elements revealed that the observed effects depended on the presence of both iron and HAs in the preparations. A decrease in the content of phosphorus in shoots and a simultaneous increase of its content in roots was noted under iron deficiency: the parameter ratio decreased from 1.2 in the control to 0.7 in the –Fe treatment. This can be indicative of the disturbance of phosphorus assimilation by plants under these conditions, because this process requires phosphorylation reactions [3], most of which are due to electron transport during the light phase of photosynthesis, whose efficiency decreases under iron deficiency, and oxidative phosphorylation during respiration. The addition of the studied preparations resulted in an increase in the ratio between the contents of iron in shoots and roots to 0.8–1.2; the maximum increase was observed for HAs, which well agrees with the data on the positive effect of HAs on oxidative phosphorylation [34].

The deficiency of iron in the nutritive medium enhanced the uptake of zinc by plants: its concentration in roots increased from 18 to 21 mg/kg. This fact well agrees with the data on the relatively low specificity of phytosiderophores released by plants of the family Poaceae under iron deficiency, which are capable of efficiently binding and transporting not only Fe^{3+}, but also Zn^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, and Co^{2+} [9]. However, our experiments showed that zinc transport to the aboveground parts of plants was disturbed under these conditions: the concentration of the element in shoots was lower than in the control in both cases. This phenomenon requires further investigations, but it may be suggested that this is related to the disturbance of the long-range transport of ions in plants. It should also be noted that the addition of HA-containing preparations to the medium resulted in an increase in the content of zinc in shoots to the control values. In view of the fact that zinc inhibits the formation of superoxide anion radical $O_2^{\bullet-}$ [7], the obtained results indicate that the positive effect of HAs and Fe–HAs under iron deficiency can be related to the improvement of the antioxidant status of plants. It is interesting to note that the maximum content of manganese in

shoots was also observed in the presence of HAs, while it was significantly lower in the treatments with the addition of Fe–EDTA and Fe–HAs. It is known that about 90% of Mn in leaves is contained in chloroplasts in the ORC system, where electrons are released from water molecules and then enter in the electron-transport chain of FSII. Consequently, it may be suggested that the positive effect of HAs on the ETR (Fig. 2b) is due to the increase in the content of ORC in their presence.

Thus, the analysis of mineral nutrient contents revealed that the addition of iron-containing preparations under iron deficiency increased the concentration of iron in plant tissues. However, the addition of Fe–EDTA increased the content of iron in both roots and shoots of plants, while the addition of Fe–HAs resulted in the predominant accumulation of iron in plant roots. This indicates the limited translocation of iron in the form of its HA complexes. On the other hand, the addition of HA-containing preparations significantly increased the input of zinc into plants, in contrast to the addition of Fe–EDTA. The obtained results indicate that the positive effect of HAs and their iron complexes under iron deficiency can be related to the increased input of zinc involved in the antioxidant protection system, which protects plants against excess free radicals. Lipids are the first targets of free radicals; therefore, the effect of the preparations on the lipid profiles of plants were then studied.

An analysis of the lipid profiles of wheat plants showed that polar lipids included monogalactosyldiacylglyceride (MGDG), digalactosyldiacylglyceride (DGDG), glucosylceramide (GC), phosphatidylethanolamine (PE), phosphatidic acid (PA), sulfoquinovosyldiacylglycerol (SQDG), phosphatidylcholine (PC), and phosphatidylglycerol (PG). Phosphatidylinositol, lysophosphatidylcholine, and lysophosphatidylethanolamine were detected in trace amounts (<0.05 mg/g). Neutral lipids included triacylglycerols (TAGs), diacylglycerols (DAGs), free fatty acids (FFAs), and sterols (St). Under iron deficiency, the total content of polar lipids in the nutritive medium decreased by 30% and that of nonpolar lipids decreased by 20% (Fig. 3), which well agrees with the earlier data demonstrating a decrease in the content of lipids under iron chlorosis conditions [25].

Under iron deficiency, the greatest decrease (by 58%) was observed for PC: a membrane lipid, precursor for MGDG synthesis, and one of the main lipid components in thylakoid membranes (Fig. 3a). It was shown that the insufficient MGDG synthesis decreases the ETR, which is due to the damage and, hence, malfunction of thylakoid membranes [11]. In addition, a significant decrease was observed for the contents of polar lipids such as PG (39%), MGDG (37%), and SQDG (32%), which are the main lipids in thylakoid membranes of chloroplasts [31]. The decrease in their content should result in the disturbance of photosynthesis in iron-deficient plants, which well agrees with the earlier revealed decrease in the content of chlorophyll and the ETR. The role of PG in the binding of proteins necessary for the functioning of the manganese cluster of ORC was also revealed earlier [30].

The addition of the iron-containing preparations Fe–EDTA and Fe–HAs resulted in a significant increase in the content of PC. In addition, an increase in the content of SQDG was revealed at the addition of Fe–EDTA, as well as an increase in the content of PG at the addition of Fe–HAs. The most manifested effect of the studied preparations was observed at the addition of HAs, when an increase in the contents of all polar lipids was noted. The obtained results well agreed with the data on the distribution of added tritium-labeled HAs from plants [23]. A hypothesis about the direct involvement of humic substances in the biosynthesis of lipids may be advanced. This hypothesis is confirmed by the presence of fatty acids (the main precursors of polar lipids in plant metabolism) in humic substances [26]. It may be suggested that some functions of HAs or their transformation products serve as precursors of lipids during their biosynthesis in plants. On the other hand, the increase in the content of polar lipids can also be due to the increase in the antioxidant status of plants in the presence of HAs under these conditions, as indicated by the minimum content of flavonoids (Fig. 2d) and the enhancement of zinc uptake. Further studies are necessary to confirm the above suppositions.

The effect of iron deficiency on the content of neutral lipids was less manifested: a reliable decrease in the content of lipids in the –Fe treatment was revealed only for FFAs (Fig. 3). Fatty acids in plants are mainly synthesized in chloroplasts; therefore, a relationship between the decrease in the content of chlorophyll and that of fatty acids is obvious (Fig. 2). On the other hand, fatty acids are the main initial compounds for the synthesis of lipids; therefore, the decrease in their content in the –Fe treatment well agrees with the above-noted decrease in the content of polar lipids. The addition of Fe–EDTA resulted in a pronounced increase in the content of FFAs; this can indicate an enhancement of lipid exchange in the given case. It is interesting to note that no analogous effect was noted at the addition of Fe–HAs: the increase in the content of FFAs from 0.10 to 0.15 mg/g was statistically insignificant. The higher effect of Fe–EFTA on the content of the main neutral lipids, as in the case of polar lipids, can be due to the high content of iron involved in metabolism in this case. It is interesting to note that the addition of all studied preparations resulted in an increase in the content of DAG, a reserve lipid, which is also a precursor of lipids entering into membranes of thylakoids (MGDG, DGDG, SQDG, and PG). It may be suggested that this indicates the activation of lipid metabolism. As in the case of polar lipids, the maximum content of neutral lipids was observed at the

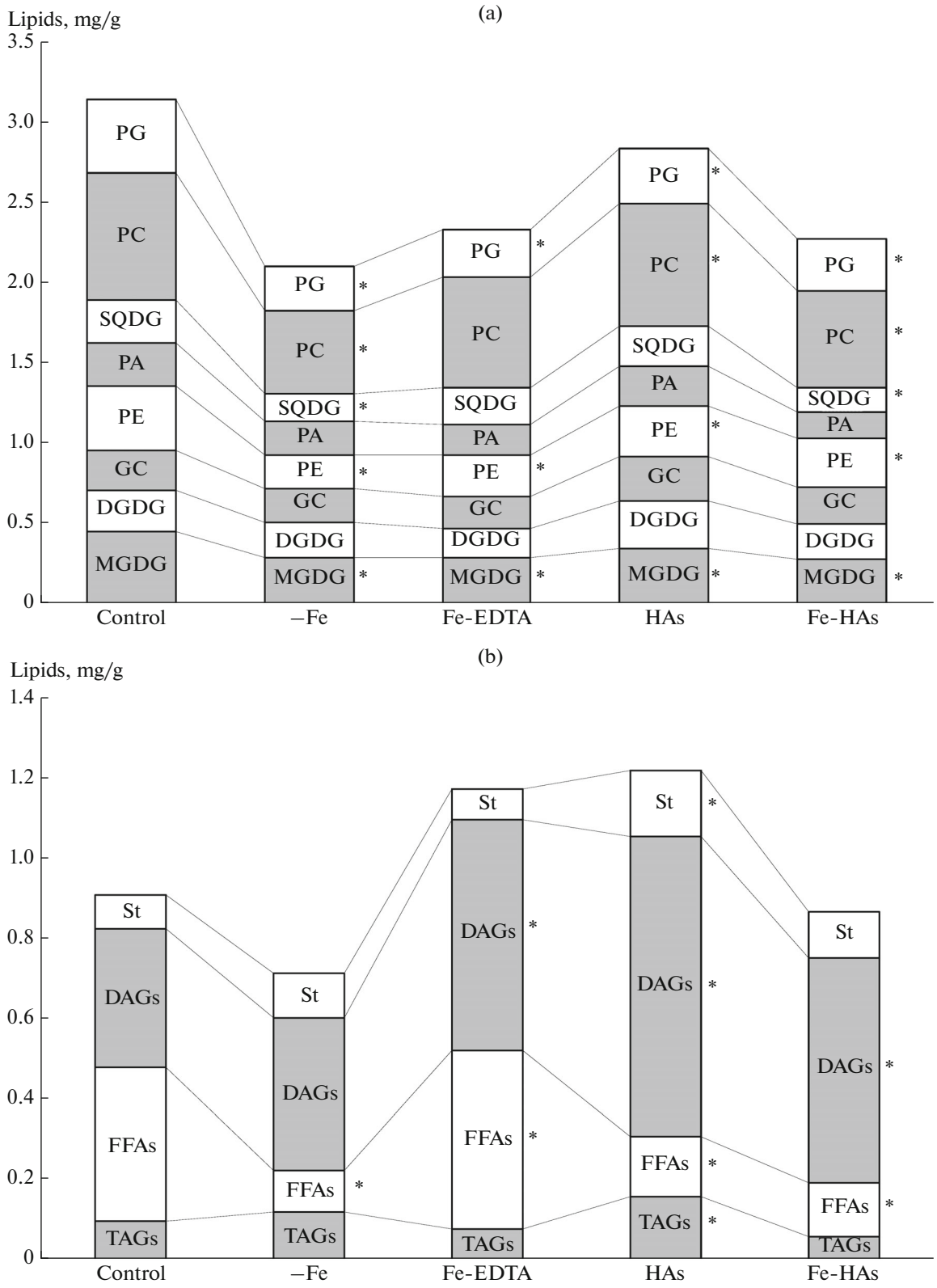

Fig. 3. Effect of iron-containing preparations and HAs on the content and composition of (a) polar and (b) neutral lipids; polar lipids: (MGDG) monogalactosyldiacylglyceride; (DGDG) digalactosyldiacylglyceride; (GC) glucosylceramide; (PE) phosphatidylethanolamine; (PA) phosphatidic acid; (SQDG) sulfoquinovosyldiacylglycerol; (PC) phosphatidylcholine; (PG) phosphatidylglycerol; neutral lipids: (TAGs) triacylglycerols; (FFAs) free fatty acids; (DAGs) diacylglycerols; (St) sterols.

addition of HAs. An exception was provided by FFAs, whose maximum content was revealed at the addition of iron in the form of EDTA complex.

Of special interest is the revealed increase in the content of sterols in the presence of HAs. It is known that almost all sterols in plants are precursors of brassinosteroids, plant hormones directly affecting the structure and functioning efficiency of thylakoids [22]. It was shown that the decrease in photosynthesis efficiency caused by elevated temperatures and oxidative stress could be compensated by the treatment with brassinosteroids [18, 29]; the involvement of brassinosteroids in the functioning of ORC was suggested [22]. Hence, the increased content of sterols in the presence of HAs under iron deficiency well agrees with the data on the enhanced uptake of manganese under these conditions.

Thus, the comparative analysis of lipid profiles showed that iron deficiency in the nutrient medium results in a decrease in the contents of both polar and neutral lipids. At the addition of iron-containing preparations, lipid metabolism is activated mainly due to the enhancement of PC synthesis. The addition of HAs under iron deficiency increases the contents of all lipid fractions, which points to the effect of HAs on lipid metabolism.

In summary, we may suggest that the positive effect of Fe–HAs on plants under iron deficiency is due to a combination of factors, the most noteworthy of which include not only the input of iron into plants, but also the effect of HAs on the antioxidant status of plants and their lipid metabolism.

CONCLUSIONS

(1) The possibility of iron input into plants from Fe–HA complexes is shown. An analysis of iron content in plant roots and shoots revealed the accumulation of the element in roots, which can be due to the predominant adsorption of Fe–HAs on the surface of roots at the limited translocation to the aboveground parts of plants.

(2) The addition of Fe–HAs under iron deficiency has a positive effect on the photosynthetic activity of plants: an enhancement of chlorophyll synthesis is observed due to the input of iron into plants, as is an enhancement of electron transport from FSII due to the humin component.

(3) The addition of Fe–HAs and HAs under iron deficiency enhances the input of Zn into plants, which can favor an increase of their antioxidant status and, hence, a decrease in signs of iron chlorosis.

(4) The addition of HAs under iron deficiency results in an increase in the contents of both polar and neutral lipids, which is indicative of the effect of HAs on their metabolism.

(5) From the obtained results, is was suggested that the positive impact of HA complexes with iron on plants under iron deficiency is due to several factors, the most important of which include the input of iron into plants, the increase of the antioxidant status of plants, and the activation of their lipid metabolism.

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