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Fullerenol affects maize plants depending on their iron status

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Abstract

Although fullerene (C_{60}) has attracted great interest as a carbon-based nanomaterial with unique properties, today, little is known about the interaction of its water-soluble derivates, including fullerenol with higher plants. Here, we investigated how fullerenol [$C_{60}(OH)_{22-24}$] affects *Zea mays*, as a Strategy II plant, depending on its iron status. Iron deficiency chlorosis is a common nutritional disorder affecting plants. Maize plants were grown hydroponically, either with [$+Fe^{II}$ (ferrous) or $+Fe^{III}$ (ferric)] or in Fe-free ($-Fe^{II}$ and $-Fe^{III}$) nutrient solution and with or without a fullerenol supply. Fullerenol affected plants differently depending on their Fe status. The beneficial effects of fullerenol were observed in the Fe^{II}-deprived plants, including successful suppression of plant Fe-deficiency chlorosis mainly in the younger (basal and middle) region of the leaf blade. This region expressed more severe chlorosis as compared with the older (apical) region of the leaf blade. These changes were accompanied by a significant increase in leaf active Fe and lowering the root apoplastic Fe, suggesting that fullerenol may enhance Fe mobilization in the roots, helping to alleviate Fe deficiency chlorosis. By contrast, there were no observable effects in the Fe^{III}-deprived plants being significantly lower in the root apoplastic Fe as compared with the Fe^{II}-deficient plants. Additionally, fullerenol did not affect the Fe-sufficient plants, irrespective of the Fe species (Fe^{III}-EDTA or Fe^{II}-EDTA) used as Fe-sources. Our results provide new evidence for the beneficial role of Fe-fullerenol interactions in the enhancement of gramineous plant tolerance to Fe deficiency conditions, which are one of the major limiting factors for crop production all over the world.

Keywords: chlorosis, fullerenol, iron deficiency alleviation, maize, *Zea mays*.

Introduction

Iron (Fe) is essential for plants as a co-factor of enzymes of key metabolic processes including respiration and photosynthesis (Marschner 1995). Iron is an element abundant in the earth's crust. However, at high pH and high bicarbonate content of calcareous soils, the availability of Fe to plants is often reduced. The deficiency of bioavailable Fe leads to a characteristic chlorotic phenotype that begins to develop in the youngest leaves. Iron deficiency chlorosis is a common nutritional disorder affecting plants and one of the major limiting factors for crop production in many areas of the world (Vose 1982, Alloway 2008).

To maintain Fe homeostasis, plants have evolved mechanisms to acquire Fe under conditions of limited availability. Maize, like other Fe-deficient grasses, respond to Fe deficiency through the so-called Strategy II, which includes *1*) the release of phytosiderophores (PSs) for chelate Fe^{III} (ferric) ions in soil and *2*) the induction of a transporter specific for Fe^{III}-PS complex in the root cell plasma membrane (Römheld and Marschner 1986). Plant PSs belong to the mugineic acid (MA) family of chelators (Hell and Stephan 2003). Both reactions of this chelation-based strategy enhanced in response to Fe deficiency are directed to improve Fe uptake. In maize, the *Yellow Stripe 1* (*YS1*) gene encoding Fe^{III}-PS transporter was firstly identified by Curie *et al*. (2001). It has been suggested that the maize YS1 (ZmYS1) is involved in both primary Fe acquisition and intracellular transport of Fe and other metals

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Abbreviations: CBNMs - carbon-based nanomaterials; EDTA - ethylenediaminetetraacetic acid; Chl - chlorophyll, ENMs - engineered nanomaterials; SPAD - spectral plant analysis diagnostic.
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(Ueno *et al.* 2009). Additionally, in maize, Fe deficiency can stimulate the release of glutamate, glucose, ribitol, and citrate from Fe-deficient roots, suggesting that the release of nonspecific root exudates may represent an adaptation to nutrient deficiency (Carvalhais *et al.* 2011). Although grasses are thought to be less sensitive to Fe deficiency than the so-called Strategy I plants (all dicots and monocots with the exception of grasses), chlorosis can also affect grasses, including maize, at high soil pH (Nordquist *et al.* 1992).

Significant progress has been made in the development of remediation techniques and crop management strategies to prevent Fe deficiency in crops (Zuo and Zhang 2011). The application of Fe fertilizers seems to be the most efficient practice to control Fe deficiency in crops. However, the supply of synthetic Fe^{III}-chelates in the soil is very costly (Fernández *et al.* 2006). Moreover, plant availability of Fe is determined by its solubility rather than by its abundance (Guerinot 2001). Therefore, approaches need to be developed to increase Fe uptake by plant roots and its transfer to shoots.

To alter plant production, the application of nanotechnology in agronomic practice has potential. It has been reported that engineered nanomaterials (ENMs) with their unique physicochemical properties (small surface area, atypical surface structure, enhanced reactivity) can be efficient for the controlled release of agrochemicals, thereby leading to an increase in nutrient utilization and crop yield (Wang *et al.* 2016, Khan *et al.* 2017). Among ENMs, carbon-based nanomaterials (CBNMs) exhibit a wide range of novel features as promising materials for numerous applications. The most investigated CBNMs are fullerene and fullerenol (F), $C_{60}(OH)_x$, x = 18-36 (Kroto *et al.* 1985, Zaytseva and Neumann 2016, Semenov *et al.* 2017). Native fullerene is insoluble in water, therefore its water-soluble derivates have been synthesised. These fullerene derivates retain the unique properties of native fullerene, which caused their wide application in biology (Partha and Conyers 2009, Prylutska *et al.* 2012, Semenov *et al.* 2017).

Although fullerene C_{60} has attracted great interest, today little is known about the interaction of its water-soluble derivates including fullerenol in higher plants. Seedlings of *Arabidopsis thaliana* treated with fullerenol showed longer hypocotyls (Gao *et al.* 2011). Fullerenol enhanced plant growth and content of compounds used in medicine of *Momordica charantiai* (Kole *et al.* 2013). Moreover, fullerenol stimulated elongation of *Hordeum vulgare* roots, especially under stressful conditions: salt stress, ultraviolet (UV)-B radiation, and the excess of salicylic acid (Panova *et al.* 2016). Foliar application of fullerenol alleviated drought impact in sugar beets (Borišev *et al.* 2016) and chlorosis in cucumber when fullerenol was used in complex with a Fe-source (Bityutskii *et al.* 2020). Very recently, we have reported that fullerenol can protect cucumber against Fe deficiency through increased utilisation of Fe in the root apoplast (Bityutskii *et al.* 2021). However, there is still incomplete information about the effects of fullerenol on Fe nutrition of gramineous plants (including maize). Their mechanisms of Fe mobilization (Strategy II) are totally different from that of Strategy I plants. Whereas the so-called Strategy I plants (dicotyledonous and non-graminaceous monocotyledonous species) reduce Fe before uptake, the Strategy II plants (grasses) respond to Fe deficiency through the chelation-based strategy, as noted above.

For that reason, the objective of this study was to investigate how fullerenol affects maize plants depending on their Fe status (Fe-sufficient and Fe-deficient plants), with special emphasis on plant-fullerenol interactions with various Fe species (Fe^{III} and Fe^{II}) used for Fe supply.

Materials and methods

Fullerenol synthesis and identification: Fullerenol ($C_{60}(OH)_{22-24}$) was synthesized and identified, as described recently (Semenov *et al.* 2011, Podolsky *et al.* 2019, Bityutskii *et al.* 2020). The following characterization data of the used fullerenol were summarized. FTIR: 3418 cm^{-1} (νO–H), 1597 cm^{-1} (νC=C), 1370 cm^{-1} (δ_SC–O–H) and 1060 cm^{-1} (νC–O). Data of experimental elemental analysis: (C: 63.72 %; H: 2.22 %), calc.: (C: 63.83 %; H: 2.13 %). A relative molar mass of 1 128 g mol^{-1} was considered in all further calculations [corresponds to $C_{60}(OH)_{24}$]. The hydrodynamic diameters and ζ-potentials of associates in binary [$C_{60}(OH)_{22-24}$–H_2O] system were -21 mv and -30 mv, respectively. Thus, even at low concentrations (1 mg dm^{-3}), aqueous fullerenol solutions were associated and electrokinetically stable (Bityutskii *et al.* 2020).

Plants and growth conditions: Maize (*Zea mays* L., cv. Malka M) seeds obtained from the Vavilov Research Institute, Plant Genetic Resources (Saint Petersburg, Russia) were germinated between two sheets of filter paper moistened with distilled water in the dark and 28 ºC for 4 d. Then, the seedlings were pre-incubated in a complete nutrient solution containing [mM]: 1.0 KCl, 3.0 $Ca(NO_3)_2$, 0.5 $MgSO_4$, 1.0 KH_2PO_4, and [μM]: 1.0 $MnSO_4$, 1.0 $ZnSO_4$, 0.5 $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$, and 10 H_3BO_3. Iron (Fe) was supplied as Fe^{III}-EDTA or Fe^{II}-EDTA at 100 μM.

After 7 d of pre-culture, the seedlings were transferred to 1 dm^3 plastic pots (three plants per pot) and exposed for following 7 d to the same nutrition solution, either with $+Fe^{III}$ and $+Fe^{II}$ supply (+Fe) or in Fe-free (-Fe) nutrient solution (*i.e.* completely without any Fe supply), and with or without fullerenol supply. Fullerenol was freshly prepared in distilled water and used at final concentrations of 0 (F0), 1 (F1), and 2 (F2) mg dm^{-3}, respectively. The pH was adjusted to 6.0. Every 2 - 3 d, the nutrient solutions were completely renewed. Plants were grown in the following conditions: day/night temperatures of 24/20 ± 2 ºC, a 16-h photoperiod, a photon flux density of 200 μmol m^{-2} s^{-1} at plant height, and air humidity of 70 %.

Spectral plant analysis diagnostic (SPAD) measurement and growth analysis: The chlorophyll (Chl) content in leaves from different positions (from the base to the youngest leaves: L3 and L4) was estimated non-

destructively as SPAD units, using a portable Chl meter (*SPAD-502*, *Minolta*, Osaka, Japan). The Chl of first (L1) and second (L2) leaves expanded during the pre-culture with Fe (+Fe) was not monitored. In maize, the Chl content along the leaf is highly heterogeneous (Repka and Jureková 1981). Therefore, the Chl analysis was focused on three regions of leaf blades: basal, middle, and apical. We measured four replicates of each leaf section per pot. At harvest, plants were divided into the following parts: roots and shoots. Samples were oven-dried at 70 ºC, then weighed and pulverized in a ceramic grinder.

Determination of active Fe in leaves and root apoplastic Fe: Active iron was determined using 2.2′-bipyridyl (83 mM) at pH 3.0 (HCl) (Abadía *et al.* 1984). At the end of the experiments, the absorbance was measured at 520 nm after the leaves (L3, 1 g) were incubated with the reagent for 24 h.

Root apoplastic Fe was determined by the method of Bienfait *et al.* (1985). At first, intact roots of each plant were washed for 10 min in a solution containing 0.5 mM $CaSO_4$ and 5 mM morpholineethanesulfonic acid (MES; pH 5.5). Then they were incubated in a solution containing 5 mM MES (pH 5.5), 0.5 mM $CaSO_4$, and 1.5 mM 2.2′-bipyridyl for 10 min under reductive conditions. These conditions were created by adding 0.5 g of solid sodium dithionite under continuous N_2 bubbling through the solution. The absorbance of apoplastic Fe in form of a red Fe^{II}-bipyridyl$_3$ complex was measured at 520 nm.

Elemental analysis: Dry leaf materials (0.1 g) were microwave-digested (*Minotavr-2*, *Lumex*, Saint Petersburg, Russia; *MDS-10*, *Sineo Microwave Chemistry Technology Co.*, Shanghai, China) in concentrated HNO_3. The content of micronutrients (Fe, Zn, Mn, and Cu) and macronutrients (P, K, and S) were determined by inductively coupled plasma optical emission spectroscopy (*Shimadzu ICPE-9000*, Kyoto, Japan).

Statistical analysis: Data were statistically evaluated by analysis of variance procedures (*Type III ANOVA*), using *IBM SPSS Statistics* (*v. 26*). Data are expressed as means ± standard deviations. Means were compared by the Student-Newman-Keuls post-hoc test at $P < 0.05$. Four replicate pots were used per treatment. To test whether investigated parameters were correlated, Pearson's coefficient (r) was determined.

Results

Plants grown in Fe-sufficient conditions exhibited different Chl content depending on Fe species used for Fe supply. Indeed, leaves at a different position (L3 and L4) of the $+Fe^{III}$ plants showed 1.4 - 1.6-fold lower SPAD compared with $+Fe^{II}$ plants, irrespective of leaf region (Fig. 1). A lack of Fe in a nutrient solution for 7 d induced severe chlorosis symptoms and the lowest SPAD units, with

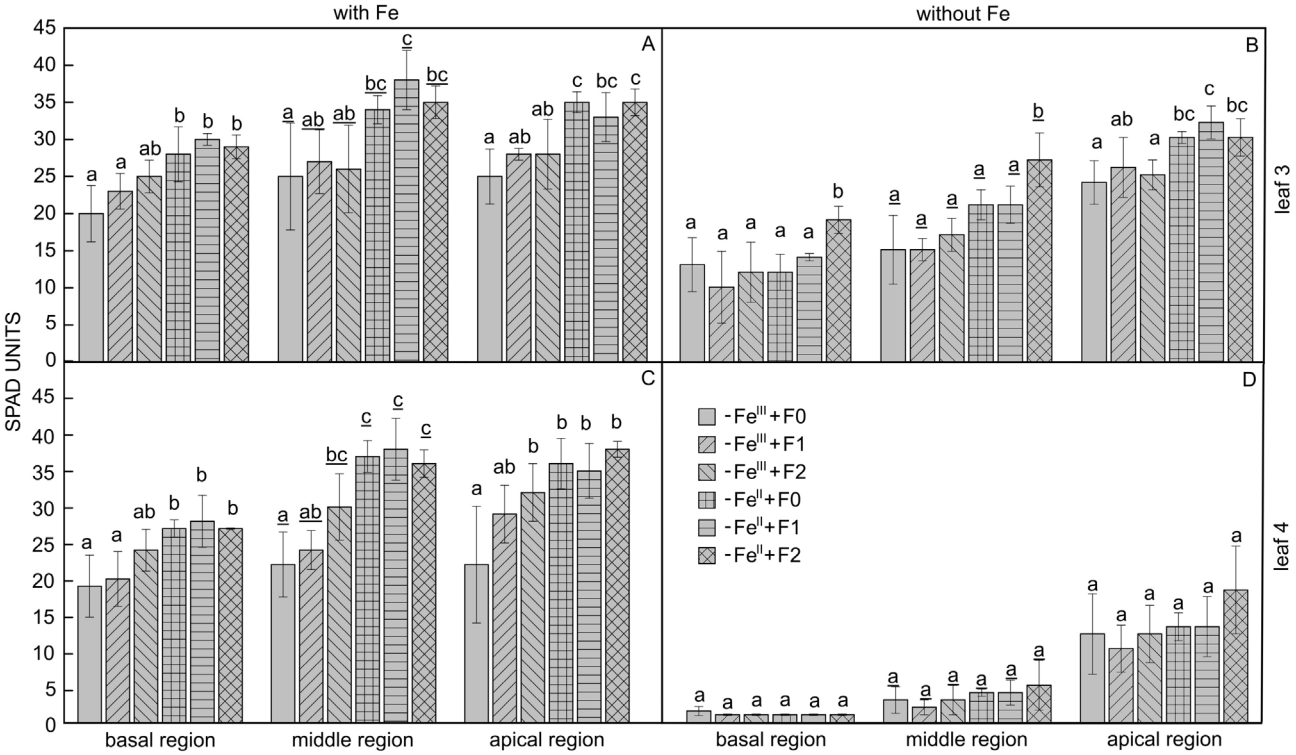

Fig. 1. SPAD units in maize leaves at different positions and blade regions grown hydroponically in a nutrient solution, either with ($+Fe^{II}$ and $+Fe^{III}$) (*A*, *C*) or in Fe-free ($-Fe^{II}$ and $-Fe^{III}$) (*B*, *D*) nutrient solution, with or without the supply of 0 (F0), 1 (F1), and 2 (F2) mg dm^{-3} fullerenol for 7 d. Means ± SDs, $n = 4$; significant differences between treatments ($P < 0.05$) are indicated by different letters.

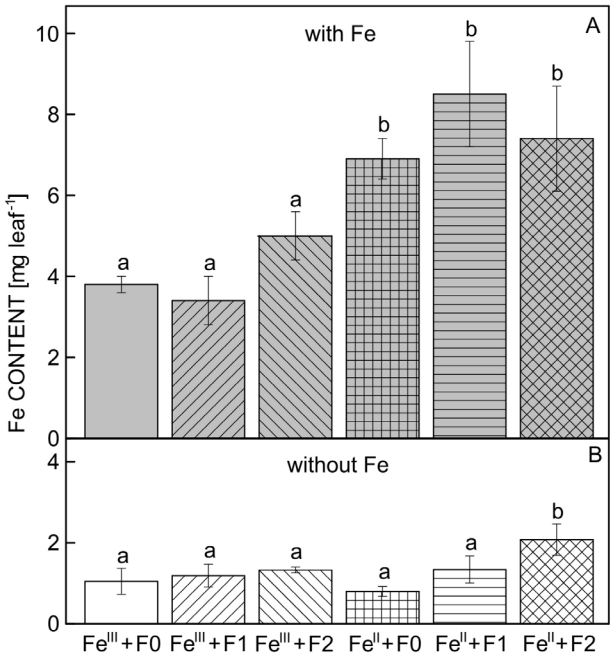

Fig. 2. Active Fe content in the third leaves (L3) of maize grown hydroponically in a nutrient solution, either with (+Fe^{II} and +Fe^{III}) (*A*) or in Fe-free (-Fe^{II} and -Fe^{III}) (*B*) nutrient solution, with or without the supply of 0 (F0), 1 (F1) and 2 (F2) mg dm^{-3} fullerenol for 7 d. Means ± SDs, $n = 4$; significant differences between treatments ($P < 0.05$) are indicated by different letters.

symptoms being more pronounced in basal and middle parts of maize blades (Fig. 1). Thus, the youngest regions of the blades showed Fe deficiency symptoms of chlorosis, while the oldest apical parts stayed green. In contrast to Fe-sufficient plants, the effect of Fe species on leaf SPAD after Fe removal was not significant except for apical parts of L3 which were formed during the pre-incubation period of +Fe supply (Fig. 1).

The fullerenol treatments at the maximal dose (F2) successfully increased SPAD values in third leaves (L3) of the -Fe^{II} plants, but not of the -Fe^{III} plants (Fig. 1). Moreover, the mutual influences of fullerenol particles were prominent in the basal (+58 %) and middle (+28 %) regions of maize blade showing severe chlorosis. However, the differences between the -Fe^{II} and -Fe^{III} plants at position L3 disappeared at position L4. Also, no distinct effects of fullerenol on leaf Chl were found in Fe-sufficient plants (Fig. 1). The effect of Fe species and fullerenol on dry biomass of maize plants grown in experimental solutions for 7 d was not significant, irrespective of the Fe supply (Fig. 1 Suppl.).

At the end of the experiment, the third leaves of the +Fe^{II} plants showed a 1.8-fold higher content of extractable (active) Fe than the third leaves of the +Fe^{III} plants – at a concentration of fullerenol F0 (Fig. 2). At the same time, Fe-deficient plants exhibited from 3.6- to 8.6-fold lower content of active Fe than Fe-sufficient plants, the effects of different Fe species on this index (active Fe) were not significant. The addition of fullerenol (F2) significantly increased (by 2.6-fold) the active Fe in chlorotic leaves (L3) of the -Fe^{II} plants, but not of the -Fe^{III} plants (Fig. 2).

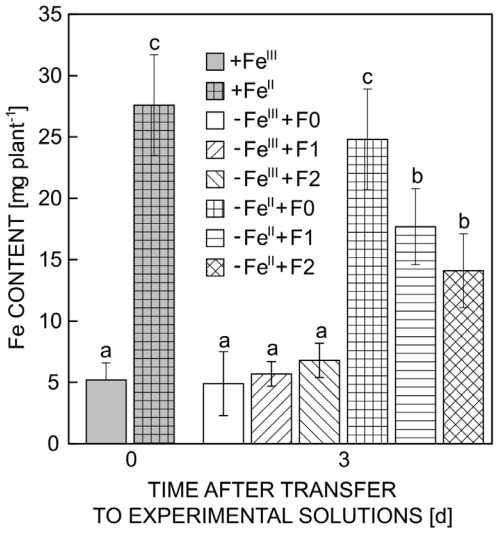

Fig. 3. Content of root apoplastic Fe of Fe-deficient maize grown hydroponically in a nutrient solution with or without the supply of 0 (F0), 1 (F1), and 2 (F2) mg dm^{-3} fullerenol for 3 d. Means ± SDs, $n = 4$; significant differences between treatments ($P < 0.05$) are indicated by different letters.

The active Fe content of the L3 positively correlated with their SPAD values: in basal region $r = 0.957$, $P < 0.01$; and in middle region $r = 0.926$, $P < 0.01$. By contrast, the +Fe plants were not affected by fullerenol treatments in terms of leaf active Fe (Fig. 2).

After Fe pre-treatment without fullerenol for 7 d, the root apoplastic Fe content of the +Fe^{II} plants was higher by 5.3-fold compared to the +Fe^{III} plants (Fig. 3). During 3 d of Fe-deficiency stress, maize roots of both the -Fe^{III} and -Fe^{II} plants did not show a significant reduction in the apoplastic Fe (Fig. 3). At the same time, fullerenol supply especially at a maximal dose (F2) significantly lowered root apoplastic Fe in the -Fe^{II} plants and did not affect that in the -Fe^{III} plants (Fig. 3).

At the end of the experiment, the total leaf Fe content of the +Fe^{II} plants was significantly higher (by 1.3- and 2.0-fold, respectively) as compared with the +Fe^{III} plants (Table 1). By contrast, the leaf content of some nutrients (Mn, Cu, P, and K) in the +Fe^{II} plants were from 1.2- to 1.8-fold lower than in the +Fe^{III} plants. At the same time, Fe species did not affect the leaf content of these nutrients in the +Fe plants (Table 1). Overall, fullerenol did not significantly alter the leaf content of all investigated nutrients in Fe sufficient plants (Table 1). After Fe removal, the differences in leaf content of macro- and micronutrients of the Fe^{III} and Fe^{II} pre-treated plants disappeared with exception of S. The concentration in the -Fe^{III} plants was a little higher than in the -Fe^{II} plants (Table 2). Additionally, the fullerenol treatment did not significantly alter the leaf content of all nutrients in Fe-deprived maize plants (Table 2).

Discussion

We assumed that fullerenol can affect maize plants

Table 1. Content of micro- and macronutrients in leaves of maize grown hydroponically in a nutrient solution with (+Fe^{II} and +Fe^{III}) Fe supply, either with or without the supply of 0 (F0), 1 (F1), and 2 (F2) mg dm^{-3} fullerenol for 7 d. Means ± SDs, $n = 4$; significant differences between treatments ($P < 0.05$) are indicated by different letters.

Treatments	Fe	Zn	Mn	Cu	P	K	S
[μg g^{-1}(d.m.)]					[mg g^{-1}(d.m.)]		
+Fe^{III} + F0	56 ± 5a	27 ± 2a	77 ± 13b	9.4 ± 1.8b	15 ± 2c	45 ± 3b	1.9 ± 0.1a
+Fe^{III} + F1	53 ± 5a	24 ± 6a	67 ± 17b	6.0 ± 2.1a	13 ± 1bc	47 ± 2b	1.8 ± 0.2a
+Fe^{III} + F2	56 ± 2a	29 ± 5a	65 ± 15b	5.8 ± 0.9a	11 ± 1ab	48 ± 6b	1.8 ± 0.1a
+Fe^{II} + F0	73 ± 5b	31 ± 4a	41 ± 6a	5.3 ± 0.6a	10 ± 2ab	36 ± 3a	1.8 ± 0.1a
+Fe^{II} + F1	81 ± 6b	26 ± 4a	34 ± 5a	4.3 ± 1.1a	9 ± 1a	32 ± 5a	1.7 ± 0.1a
+Fe^{II} + F2	72 ± 6b	27 ± 1a	43 ± 8a	4.2 ± 0.4a	10 ± 1ab	39 ± 4ab	1.7 ± 0.1a
[μg $plant^{-1}$]					[mg $plant^{-1}$]		
+Fe^{III} + F0	12 ± 1a	8 ± 2a	18 ± 4ab	2.7 ± 1.1c	4 ± 1a	9 ± 2a	0.5 ± 0.2a
+Fe^{III} + F1	15 ± 6ab	8 ± 1a	16 ± 2ab	1.6 ± 0.4ab	3 ± 1a	14 ± 1b	0.5 ± 0.1a
+Fe^{III} + F2	18 ± 4ab	9 ± 2a	20 ± 5b	1.8 ± 0.6ab	4 ± 1a	13 ± 1ab	0.6 ± 0.2a
+Fe^{II} + F0	24 ± 4b	10 ± 1a	14 ± 3ab	1.8 ± 0.4ab	3 ± 1a	10 ± 1ab	0.6 ± 0.1a
+Fe^{II} + F1	26 ± 8b	8 ± 3a	11 ± 2a	1.4 ± 0.4a	3 ± 1a	11 ± 1ab	0.6 ± 0.2a
+Fe^{II} + F2	21 ± 5ab	8 ± 1a	12 ± 1a	1.2 ± 0.3a	3 ± 1a	11 ± 2ab	0.5 ± 0.1a

Table 2. Content of micro- and macronutrients in leaves of maize grown hydroponically in Fe-free (-Fe^{II} and -Fe^{III}) nutrient solution, either with or without the supply of 0 (F0), 1 (F1), and 2 (F2) mg dm^{-3} fullerenol for 7 d. Means ± SDs, $n = 4$; significant differences between treatments ($P < 0.05$) are indicated by different letters.

Treatments	Fe	Zn	Mn	Cu	P	K	S
[μg g^{-1}(d.m.)]					[mg g^{-1}(d.m.)]		
-Fe^{III} + F0	34 ± 4a	80 ± 6a	94 ± 12a	6.7 ± 0.7a	14 ± 1a	47 ± 3a	3.1 ± 0.4b
-Fe^{III} + F1	31 ± 2a	87 ± 11a	92 ± 10a	6.0 ± 0.6a	13 ± 2a	47 ± 3a	3.1 ± 0.2b
-Fe^{III} + F2	38 ± 10a	97 ± 16a	95 ± 13a	6.5 ± 1.1a	14 ± 1a	45 ± 2a	2.9 ± 0.2ab
-Fe^{II} + F0	34 ± 2a	72 ± 9a	83 ± 10a	5.9 ± 1.3a	12 ± 2a	45 ± 4a	2.5 ± 0.2a
-Fe^{II} + F1	33 ± 6a	78 ± 11a	97 ± 13a	5.7 ± 0.9a	13 ± 1a	45 ± 3a	2.8 ± 0.3ab
-Fe^{II} + F2	31 ± 1a	87 ± 23a	88 ± 11a	5.8 ± 0.7a	13 ± 2a	46 ± 4a	2.5 ± 0.1a
[μg $plant^{-1}$]					[mg $plant^{-1}$]		
-Fe^{III} + F0	5 ± 1a	11 ± 2a	13 ± 2a	1.0 ± 0.2a	2 ± 0.6a	7 ± 1a	0.48 ± 0.14a
-Fe^{III} + F1	4 ± 1a	12 ± 2a	13 ± 1a	0.8 ± 0.1a	2 ± 0.2a	7 ± 1a	0.44 ± 0.02a
-Fe^{III} + F2	5 ± 2a	12 ± 4a	13 ± 1a	0.8 ± 0.3a	2 ± 0.3a	6 ± 1a	0.36 ± 0.04a
-Fe^{II} + F0	4 ± 1a	10 ± 2a	11 ± 1a	0.8 ± 0.2a	2 ± 0.1a	6 ± 1a	0.33 ± 0.04a
-Fe^{II} + F1	4 ± 1a	10 ± 1a	12 ± 1a	0.8 ± 0.2a	2 ± 0.2a	6 ± 1a	0.37 ± 0.08a
-Fe^{II} + F2	5 ± 1a	13 ± 1a	13 ± 2a	0.9 ± 0.1a	2 ± 0.3a	7 ± 1a	0.39 ± 0.08a

differentially depending on their Fe status, which was created due to a different supply of Fe (+Fe or -Fe) and Fe species (ferric or ferrous Fe). Although the concentrations of Fe^{II}-EDTA and Fe^{III}-EDTA in the nutrient solutions were equal, the +Fe^{II} plants exhibited significantly higher root apoplastic Fe, leaf total Fe, and leaf active Fe than the +Fe^{III} plants (Figs. 2 and 3, Table 1). At first, Fe must be released from the synthetic Fe-chelate so the plant can absorb it. As Strategy II plants, maize roots release PSs capable of solubilizing external Fe^{III} and then transporting the Fe^{III}-siderophore complex into the cell (Ueno *et al.* 2009). In Strategy II plants, chelating agents would compete with PSs for Fe binding. Complexes of Fe-PSs are far less stable than synthetic chelates, therefore splitting of the chelate by ligand exchange may be a difficulty for the use of Fe-chelates by these plants (Lucena 2006). Complexes of Fe^{III} are known to be more stable than that of Fe^{II} (Lucena 2006), therefore maize seems to uptake Fe better from the less stable Fe^{II}-EDTA (Figs 2 and 3, Table 1). Furthermore, the Strategy II YS1 is thought to be capable of transporting not only Fe^{III} but also Fe^{II}, depending on the presented particular chelated form (Roberts *et al.* 2004). It should be noted that the more intensive transport of Fe to leaves in the +Fe^{II} plants resulted in decreased ratios of Fe with some nutrients (P, K, Zn, Cu), however, the total content of these nutrients, as well as plant growth and SPAD values, were not significantly affected by Fe^{II} treatments, at least during 14 d, as compared with Fe^{III} treatments (Table 1, Figs. 1 and 1 Suppl.). Against this background, the effect of fullerenol was distinct neither in the +Fe^{II} nor in +Fe^{III} maize plants, irrespective of fullerenol dose (Figs. 1, 2, 3 and 1 Suppl., Table 1).

The more pronounced effects of fullerenol were observed in the Fe-deprived maize plants which exhibited

typical symptoms of Fe chlorosis: low leaf SPAD values, low leaf total Fe and leaf active Fe, depressed shoot growth (Figs. 1, 2, 3 and 1 Suppl., Table 2). Fullerenol treatment at a maximal dose (F2) significantly increased leaf (L3) Chl content, and this was most distinctly observed in the younger leaf zone (basal and middle regions) of the Fe^{II}-deprived plants (Fig. 1). Although fullerenol did not affect the total leaf Fe, it significantly enhanced the leaf (L3) active Fe in the Fe^{II}-starved plants (Table 2; Fig. 2). This ameliorative effect of fullerenol was accompanied by a significant decrease in root apoplastic Fe (Fig. 3). In contrast to +fullerenol plants, such an effect was not exhibited in control plants (-Fe) without fullerenol treatments (Fig. 3). It has been reported that hydroponically growing maize was not able to mobilize its root apoplastic Fe under Fe deficit as compared with Strategy I plants (bean, *Chlorophytum*) (Bienfait *et al.* 1985). The authors suggest that mugenic acid excreted by the maize in a nutrient solution is much too dilute, and therefore inefficient in the dissolution of root Fe precipitates. Thus, fullerenol protects maize against a lack of Fe through mobilization of root apoplastic Fe, being more pronounced in the -Fe^{II} plants with the highest pool of apoplastic Fe formed during pre-incubation with Fe^{II}-EDTA. A similar effect of fullerenol was observed for cucumber – a Strategy I plant (Bityutskii *et al.* 2021), suggesting that mechanisms underlying physiological activity of fullerenol are the same either in Strategy I or Strategy II plants. Although in maize Strategy I genes were also identified, suggesting that maize may utilize a combined Fe uptake strategy (Li *et al.* 2018), the Strategy II mechanism appears to be most pronounced in this plant species. Furthermore, recent studies have shown a prominent role of fullerenol in foliar Fe fertilization of cucumber plants subjected to Fe-deficiency (Bityutskii *et al.* 2020). Interestingly, leaf penetration of Fe was expressed only when fullerenol was applied in combination with Fe^{II}-sulfate. Taken together, these results suggest that the Fe^{II}-fullerenol interactions are critical for the effectiveness of fullerenol applied to both leaves and roots.

Leaf growth of monocots is restricted to the leaf base because here cell division and expansion occur (Langer 1979). It seems the fullerenol induced Chl changes were observed in the younger zone (L3), which exhibited more intensive cell enlargement, thereby an increased Fe requirement during Fe deficiency than the apical region of maize blades. Apical parts of maize (L3) were developed predominantly under Fe-sufficient conditions. Therefore, at the end of the experiment, apical SPAD values were significantly higher and thereby less sensitive to fullerenol treatments in comparison to the basal region (Fig. 1). Interestingly, in maize leaf blades expression of *ZmYS1* is regulated by their Fe status, being 20-fold higher in Fe-stressed young leaf blades than in the oldest (Ueno *et al.* 2009). Fullerenol induced changes to the Chl content were clearly observed in L3 and did not occur in L4 (Fig. 1). The results suggest that after Fe removal the ameliorative effects of fullerenol can be limited by the size of the root apoplastic Fe pool, even -Fe^{II} plants exhibited many times larger apoplastic Fe than the -Fe^{III} plants (Fig. 3).

Little is known about mechanisms underlying the physiological activity of fullerenol in higher plants. A beneficial influence of fullerenol in mobilizing apoplastic Fe might be caused by the Fe-fullerenol interactions. Fullerenol is known to be rich in OH groups, which seem to be important for preventing oxidation of Fe^{II} and aggregation of Fe oxides in the root apoplast. Moreover, fullerenol can directly reduce Fe^{III} to Fe^{II} *via* electron transfer of fullerenol-Fe^{III} complex (Zhou *et al.* 2020). Also, carbon nanotubes may have a role in the reduction of Fe^{III} to Fe^{II} oxidation state (Tiwari *et al.* 2014). Positive ferrous ions can bind with negatively charged nanoparticles of fullerenol. As a result, fullerenol surface charge shifts to the more positive values, thereby creating a delivery system for Fe^{II} (Seke *et al.* 2019). Additionally, fullerenol might directly facilitate membrane transport of ferrous Fe in roots. Some authors believe that fullerenol is mobile in plant tissues and it has the capability for penetration through biomembranes (Kole *et al.* 2013, Borišev *et al.* 2016, Liang *et al.* 2018). Also, the beneficial effects of fullerenol nanoparticles on plants are due to their antioxidant activity, *i.e.*, an ability to serve as a scavenger of free radicals (Borišev *et al.* 2016, Panova *et al.* 2016). Further investigations are required to elucidate the biochemical mechanisms and determine the functional activity of fullerenol under Fe deficient conditions.

Conclusions

Fullerenol added in a nutrient solution could act differently depending on the Fe status of maize plants. In Fe-sufficient plants, fullerenol did not affect plants, irrespective of the Fe species (Fe^{III}-EDTA or Fe^{II}-EDTA) used, whereas in Fe-starved plants it had beneficial properties in the alleviation of Fe deficiency chlorosis. Fullerenol treatments significantly increased Chl of the youngest zone of leaf blades and leaf active Fe and were the most pronounced in the Fe^{II}-deprived plants. The beneficial effects were likely caused by a significant decrease in the root apoplastic Fe, which was larger in the Fe^{II} than in Fe^{III} pre-treated plants. There were no observable effects of fullerenol when Fe deficiency was induced by the exclusion of Fe^{III} from a nutrient solution. The results provide new evidence for the beneficial role of fullerenol in the mineral nutrition of gramineous plants based on its interactions with Fe.

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