DOI: 10.1016/j.plantsci.2020.110431

Root-shoot-root Fe translocation in cucumber plants grown in a heterogeneous Fe provision

Valentinuzzi Fabio[1], Pii Youry[1,*], Porfido Carlo[2], Terzano Roberto[2], Fontanella Maria Chiara[3], Beone Gian Maria[3], Astolfi Stefania[4], Mimmo Tanja[1], Cesco Stefano[1]

[1]Faculty of Science and Technology, Free University of Bozen-Bolzano, I-39100 Bolzano, Italy

[2]Department of Soil, Plant and Food Sciences, University of Bari ''Aldo Moro'', I-70126 Bari, Italy

[3]Department for Sustainable food process, Faculty of Agriculture, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, I-29122 Piacenza, Italy

[4]Department of Agricultural and Forestry Sciences, University of Tuscia, I-01100Viterbo, Italy

*Corresponding author:
Youry Pii

Faculty of Science and Technology, Free University of Bozen-Bolzano

Piazza Università 5, I-39100

Bolzano, Italy

Phone: +390471017164

e-mail: youry.pii@unibz.it

Abstract

Iron (Fe) is an essential micronutrient for plant life and development. However, in soil, Fe bioavailability is often limited and variable in space and time, thus different regions of the same root system might be exposed to different nutrient provisions. Few studies showed that the response to variable Fe provision is controlled at local and systemic levels, albeit the identity of the signals involved is still elusive. Iron itself was suggested as local mediator, whilst hormones were proposed for the long-distance signalling pathway. Therefore, the aim of this work was to assess whether Fe, when localized in a restricted area of the root system, might be involved in both local and systemic signaling. The combination of resupply experiments in a split-root system, the use of ^{57}Fe isotope and chemical imaging techniques allowed tracing Fe movement within cucumber plants. Soon after the resupply, Fe is distributed to the whole plant, likely to overcome a minimum Fe concentration threshold aimed at repressing the deficiency response. Iron was then preferentially translocated to leaves and, only afterwards, the root system was completely resupplied. Collectively, these observations might thus highlight a root-to-shoot-to-root Fe translocation route in cucumber plants grown on a patchy nutrient substrate.

Keywords: Split root; cucumber; Fe deficiency; ^{57}Fe isotope; Fe translocation

1. Introduction

In natural environments, soil represents the main source of mineral nutrients required for plant growth, species propagation, and crop productivity. However, nutrients are not homogeneously distributed in the different soil layers, showing variation along the soil profile as well as its horizontal axis [1]. In fact, nutrients can feature a patchy distribution [2,3], thus being more abundant in defined soil areas, often named "nutrient hotspots" [4]. Indeed, such heterogeneity in nutrients distribution can depend on the chemical and physical characteristic of the soil and on the organic inputs, followed by microbial degradation, which make resources available with different spatial and temporal dynamics as well as in different chemical forms [1]. These phenomena might also occur in cultivated soils after a chemical fertilization, particularly when approaches of precision agriculture are exploited [1]. The presence of nutrient hotspots gives rise to a broad spectrum of plant responses that affect root morphology and physiology [1,5]. Concerning morphological changes, it has been shown that plants can promote the growth of the root system, by inducing i) the elongation of individual roots [6–9], ii) the increase of the total root length [10–12] and iii) the formation of lateral roots [3,13]. On the other hand, the physiological adaptations are related to the increased ability of the plants to take up nutrients, either *via* increasing the transporters uptake capacity (*i.e.* higher V_{max}) or *via* enhancing their affinity (*i.e.* lower K_m) for the substrate [1]. Indeed, if nutrient-starved roots are locally resupplied, the uptake rate has been shown to transiently increase by about two to three times in most cases [10,14,15], even though larger increases have been observed as well [10,15,16]. Generally, the physiological changes occur before the morphological ones, thus suggesting that the provision of the nutrient might act itself as molecular signal [10,14,16].

Iron (Fe) is an essential microelement for plants since it takes part into a plethora of physiological and biochemical processes [17], being Fe able to switch between two redox states (*i.e.* Fe(II) and Fe(III)), and thus functional to electrons transport chains and to redox enzymatic reactions [18,19]. Despite being the fourth most abundant element on the Earth's crust [20], Fe is very often not bioavailable, especially in neutral and alkaline soils, thus posing serious threats to plant growth and productivity [21]. The mechanism developed by dicots to take up Fe from the growth substrate is a reduction-based process and includes three main stages: Fe(III) solubilization (rhizosphere acidification and release of chelating compounds), Fe(III) reduction to Fe(II) (through the activity of Fe Chelate Reductase -FCR- enzymes), and Fe(II) uptake *via* specific transporters [22]. Within a condition of limited micronutrient availability, the aforementioned mechanisms are significantly enhanced both at the biochemical and transcriptional level enabling plants to improve uptake fluxes [22].

So far, several pieces of research have been carried out to study the physiological effect of a heterogeneous Fe provision in dicot plants. In particular, it has been shown that the Fe transporter (IRT1) and FCR enzyme (FRO2) were controlled at both local and systemic level in *Arabidopsis thaliana* plants [23]. More recently, new insights on this process have been given by applying the split root technique in cucumber plants [24]. In fact, it was demonstrated that, even though the Fe has been resupplied to only half of the root system, the *CsFRO1* and *CsIRT1* transcripts were induced in the resupplied side of the roots, whilst they were down-regulated in the non-resupplied one [24]. These observations suggest that Fe might function as local messenger for the induction of the uptake mechanism. On the other hand, the repression of the physiological response in the non-resupplied side of the root system was ascribed either to the absence of Fe itself or to a possible systemic signal, shutting down the Fe deficiency response in order to preserve metabolic energy [24]. However, to date, the nature of the chemical signal reaching the non-resupplied side of the root system has remained poorly understood, even though the interplay among different molecular effectors, as for instance plant hormones, has been hypothesized [25].

Thus, in the present research we aimed at assessing whether Fe, if only localized in a specific and restricted area of the whole root system, might be involved in both local and systemic signaling, considering not only root-to-root signaling but also the involvement of shoots in Fe translocation and allocation. To this purpose, we used a split root approach to cucumber plants, coupled with stable labelled Fe isotope and chemical imaging techniques to trace the movement of Fe within the plants. The results are discussed considering i) the heterogeneous distribution of the nutrient availability in soils, also including cultivated soils managed with an approach of sustainable-oriented smart agriculture, and ii) the purpose to optimize the exploitation of the natural soil Fe-sources.

2. Materials and Methods

2.1 Plant material and growth conditions

Cucumber (*Cucumis sativus* L.) seeds were germinated on filter paper soaked in 0.5 mM $CaSO_4$ for 5 days in darkness and suspended vertically [26]. After germination, the plants were transferred into in 1.5 L pots with continuously aerated nutrient solutions and grown in a climatic chamber with light/dark cycle of 14/10 h and 24°C/19°C of temperature. The relative humidity was set at 70% and the light intensity was 250 µmol m^{-2} s^{-1}. The hydroponic solution was composed as follows: 2 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.1 mM KH_2PO_4, 0.1 mM KCl, 0.5 mM $MgSO_4$, 10 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.2 µM $CuSO_4$, 0.1 µM $ZnSO_4$, 0.01 µM $(NH_4)_6Mo_7O_{24}$. Iron was supplied as Fe(III)-EDTA to a final concentration of 5 µM. The pH of the nutrient solution was titrated at 6.0 with 1 M KOH. Cucumber plants were cultivated for 7 days, then the primary roots were cut in order to favour the development of lateral roots. After the root excision, plants were incubated for 16 h in a 0.5 mM $CaSO_4$ aerated solution and then cultivated for further 7 days in a nutrient solution with 5 µM Fe(III)-EDTA. At the end of the second week of cultivation, the root system was divided in two equal portions and placed in a split-root growing system, as previously described [27], and grown there for further 7 days. The system is composed by two separated compartments containing an equal volume of Fe-free nutritive solution to induce Fe deficiency condition in cucumber plants. Afterwards, cucumber plants were resupplied with 80 µM Fe(III)-EDTA in either one or both compartments. Samples were collected and analysed 12, 24 and 48 hours after the Fe resupply. A representative drawing of the experimental plan is reported in Supplementary Figure 1.

2.2 Determination of Fe chelate reductase activity in intact cucumber roots

The reduction of Fe (III)-EDTA by the root system of hydroponically grown cucumber plants was measured colorimetrically using bathophenantroline disulfonate (BPDS), as previously described [28]. The two halves of the root systems were incubated in separate jars containing the reagent solution containing 0.5 mM $CaSO_4$, 10 mM MES NaOH (pH 5.5), Fe(III)-EDTA 0.25 mM and BPDS 0.6 mM in the dark at 25°C. After 30 min incubation, the absorbance of the reagent solution was recorded at 535 nm and the amount of Fe(III) reduced was calculated on the base of the Fe(II)-$BPDS_3$ complex formed using the molar extinction coefficient of 22.1 mM^{-1} cm^{-1}.

2.3 Tracing of ^{57}Fe analysis

With the aim of tracing the movement of Fe within cucumber plants, Fe deficient split plants were resupplied in one of the two nutrient solution compartments with 80 µM ^{57}Fe(III)-EDTA. The ^{57}Fe

source was prepared starting from 100 µg g^{-1} $^{57}FeCl_3$ (ISC Science, Oviedo, Spain), which was chelated with an excess of 0.1 M EDTA and titrated to pH 6 with 1 M KOH [26].

Samples were collected 12, 24 and 48 hours after the resupply and ^{57}Fe non-specifically bound to the cell wall was removed as previously described [29]. Root and leaf tissues were separated, oven-dried at 50°C for 48 h, weighed and then digested with concentrated HNO_3 (65% (v/v), Carlo Erba) using a single reaction chamber (SRC, UltraWAVE, Milestone Inc, Shelton, CT, USA).

The mineralized solutions were spiked with 200 ppm ^{54}Fe (99.92%) standard solution (ISC Science, Oviedo, Spain) and Fe isotope ratios measurements were carried out by ICP-MS (Agilent 7900, Agilent technologies, USA) [30–32]. Fe concentration was determined by isotope dilution analysis using a collision cell ICP-MS instrument with helium as collision gas. Instrument and octapole operating condition are summarised in Supplementary Table 1.

Isotope pattern deconvolution (IPD) is the most complete mathematical approach to perform multiple-spiking Isotopic Dilution (ID) [33,34], that is needed to quantify the naturally-occurring element and two enriched tracers [35].

2.4 Micro-focused X-ray fluorescence (µ−XRF) imaging

Micro X-ray fluorescence maps were collected with a laboratory benchtop µ-XRF spectrometer (M4 Tornado, Bruker Nano GmbH, Berlin, Germany). This instrument is equipped with a micro-focus Rh X-ray source (50 kV, 600 µA), a polycapillary X-ray optics with a spotsize of 25 µm and two XFlash™ energy dispersive silicon drift detectors with 30 mm^2 sensitive area and an energy resolution of 140 eV @ Mn Kα. The two detectors, placed at opposite sites compared to the X-ray optics, allow to reduce shadowing effects in the elemental maps and obtain a better signal-to-noise ratio (S/N). Cucumber leaves were sampled and pressed between two circles of filter paper in a closed Petri dish to keep the sample flat during the freeze-drying process; i.e., the Petri dishes containing the flat leaves were quickly frozen in liquid nitrogen and then freeze-dried under vacuum. All the analyses were performed under reduced pressure (20 mbar) by acquiring one spectrum every 25 µm step, with an acquisition time of 10 ms per step. In order to increase the S/N, each sample was scanned 30 times and the spectra averaged. For each of the leaves, a rectangular area of 200 pixels (ca. 5 mm) height and 700-1200 pixels (ca. 17.5-30 mm) width (depending on leaf dimension), was selected approximately in the middle of the leaf for the analysis. XRF hyperspectral data and images were processed by PyMca 5.1.3 [36] and Datamuncher [37] software. All the maps were collected using the same analytical conditions. The same intensity scale was adopted to visualise the distributions of the same element in all the maps. Therefore, the elemental maps of the same element can be directly compared. Brighter colours in the map mean a higher concentration of the element.

In order to better evaluate the distribution of Fe over the leaf, the relative Fe amount in different leaf areas was obtained by using PyMca software (version 5.1.1). Briefly, the leaf µ-XRF map was divided into 8 identical regions (4 to the left from the central vein and 4 to the right) and the XRF sum spectrum of each region was obtained by summing all the spectra of the pixels contained in that area. The peak area of the Fe-Kα line of the sum spectra, collected in each region of the leaf, was divided by the peak area of the scatter signal (Rh-Kα), used to normalize the data for X-ray source variations [38]. Three maps were elaborated for each treatment and data averaged.

2.5 Quantitative X-ray fluorescence analysis

Quantitative Fe concentration data (expressed as µg cm^{-2}) of leaves samples were obtained by using an energy dispersive XRF spectrometer (Thermo-NITON XL3t) equipped with a Ag collimator source (spot size ca. 6 mm) operating at 50 kV and 40 µA and a large area SDD detector (160 eV resolution @ Mn Kα). A real acquisition time of 120 s was used for each point. Five points were analysed for each sample and the results averaged. Quantitative data were obtained using the Thermo-NITON instrument proprietary software (NDTR v.6.5.2) in "Thin sample (standard filter) mode". The matrix of the sample was assumed to be uniformly constituted of cellulose [39]. A cellulose filter standard (Thermo – P/N 420-026 S/N A3-1572) was used to check the instrument accuracy.

2.6 Statistical analysis

Data are expressed as means (±SE). Student's t-tests and one-way ANOVAs was carried out using GraphPad Prism version 6.00 for Mac OS X (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 Fe chelate reductase activity

The Fe chelate reductase (FCR) activity was measured in split cucumber plants at 12, 24 and 48 hours after the resupply with 80 µM Fe(III)-EDTA. In particular, a set of plants has been resupplied in both nutrient solution compartments (+/+) with Fe(III)-EDTA, a second group was resupplied only in one compartment (+/-), whereas a third set was not provided with Fe (-/-) (Figure 1).

At 12 hours after the resupply, the FCR activity was significantly increased in the (+/+) plants compared to those (-/-) (Figure 1A). Interestingly, when the Fe was furnished only in one compartment (+/-), the FCR activity strongly increased in the Fe-supplied side of the root system as compared to the untreated one, reaching values even doubled than those measured in the (+/+) plants (Figure 1A). In (+/-) plants, the same pattern of FCR activation was also maintained at 24 hours after the Fe supply in only one root compartment (Figure 1B). Differently, the (+/+) plants showed a strong enhancement of FCR, which reached the same values observed in the resupplied side of the (+/-) plants (Figure 1B). At 48 hours after the resupply, the FCR response in the (+/+) treatment was reduced (Figure 1C). Interestingly, at this time, in (+/-) plants the resupplied side still displayed a significantly higher FCR activity as compared to the untreated one; this pattern was not confirmed in (+/+) and (-/-) plants, which showed a comparable FCR activity (Figure 1C).

3.2 Monitoring of Fe translocation in split cucumber plants

Split cucumber plants, previously grown under Fe deficiency (-/-) as described above, were resupplied with 80 µM ^{57}Fe-EDTA in one of the two compartments, in order to trace the movement of the micronutrient in the different plant tissues within 48 hours. The analysis of both the natural Fe (^{56}Fe) and the stable isotope (^{57}Fe) was carried out by the isotope dilution method, followed by the isotope pattern deconvolution approach [26].

As shown in Figure 2A, the concentration of ^{57}Fe showed a statistically significant increase in the ^{57}Fe-resupplied roots already at 12 h after the treatments. The same increasing trend was also maintained at 24 h after the treatment, whereas ^{57}Fe concentration did not change afterwards (Figure 2A). Interestingly, the analyses of the root portion not supplied with the Fe stable isotope revealed that, 12 h after the resupply, the ^{57}Fe concentration detected in the root tissue was statistically higher as compared to the natural abundance of the isotope found in the control plants (Figure 2A). The ^{57}Fe concentration in not resupplied roots remained constant at 24 h after the treatments, whereas it statistically increased at 48 h, revealing a slightly different trend when compared to the roots of the resupplied compartment (Figure 2A).

The dynamics of ^{56}Fe concentration in the roots after the resupply were also studied (Figure 2B). Although no significant difference could be highlighted between control (-/-) and resupplied plants at 12 h after the treatment, the concentration of ^{56}Fe showed a strong decrease at both 24 and 48 h in the resupplied root tissue (Figure 2B). The same significant trend was also observed in not resupplied roots, albeit less dramatic (Figure 2B).

Following a similar profile as observed in resupplied roots, the concentration of ^{57}Fe in cucumber leaves showed a significant increase at each analysed time, albeit to different extent with respect to the previous sampling time point (Figure 2C). In fact, at 12 h after treatments a 6-fold increase in the ^{57}Fe concentration was detected in comparison to control (-/-) plants; similarly, at 24 h a 10-fold enhancement was observed with respect to the 12 h sampling time (Figure 2C). On the other hand, only a 40% increase in ^{57}Fe concentration was detected in leaves of plants resupplied for 48 h with respect to those treated for 24 h (Figure 2C). Concerning the concentration of ^{56}Fe, no significant alterations have been observed over the time in the leaves of treated plants as compared to control (Figure 2D).

3.3 Fe allocation dynamics in the leaves

In order to understand whether the resupply of a single portion of the root system (+/- plants) could influence the distribution of Fe in the leaves in comparison to fully resupplied (+/+) plants, Fe distribution maps were acquired by μ-XRF at 12, 24 and 48 h after the treatments (Figure 3A). As shown in Figure 3A, the resupplied (+/-) plants showed, over the time, an increasing content of Fe, which distributed homogeneously in the leaves. The uniform distribution of Fe in the leaves is better visible in Figure 4, where the relative amount of Fe was determined in different leaf areas. These data suggested that the not uniform supply of the micronutrient at root level do not imply an unbalanced Fe distribution at leaf level. The Fe concentration during the resupply of (+/-) plants was also determined by XRF and compared to that of not resupplied (-/-) and fully resupplied (+/+) plants (Figure 3B). Interestingly, at 12 h after the treatment, the total Fe concentration did not show any significant alteration, independently from the treatment (Figure 3B), confirming the data obtained with the isotope dilution method (Figure 2D). At 24 h hours after the treatments, a significant nut moderate increase in the Fe concentration was observed in both (+/+) and (+/-) leaves, even though the highest content was detected in the latter (Figure 3B). On the contrary, the highest Fe concentration was detected in (+/+) plants at 48 h, whereas the leaves of (+/-) plants showed a slightly lower micronutrient content (Figure 3B), as also visible in the Fe distribution maps (Figure 3A). The concentration of Fe in (+/-) leaves after 48h was much higher than in (-/-) leaves after the same period.

Beside Fe, also zinc (Zn) and calcium (Ca) showed a different distribution at leaf level in (+/-) plants after Fe resupply (Figure 5). In particular, Zn displayed a redistribution over time; at 12 h, it was mainly located in the main veins, whereas at 24 h it appeared in both principal and secondary veins. At 48 h, Zn appeared diffused all over the whole leaf area (Figure 5A).

Calcium (Ca) was mostly located in trichomes of cucumber leaves, irrespectively from the treatments, albeit at 48 h it showed a higher concentration in (+/+) plants with respect to (-/-). Interestingly, in (+/-) plants, Ca showed the same distribution pattern as in controls, even though the highest concentration in the trichomes was shown at 24 h after the resupply (Figure 5B); a decrease in Ca concentration was observed afterwards.

The distribution of the other elements detected by µXRF (*i.e.* phosphorus, sulfur, potassium, manganese and copper) was not significantly different between (+/-) plants at different times after resupply, also when compared to (+/+) and (-/-) plants. ~~'~~As an example, ~~(d~~data ~~not shown).~~for Mn and Cu are reported as Supporting Material.

ha formattato: Evidenziato

ha formattato: Evidenziato

ha formattato: Evidenziato

4. Discussion

Iron (Fe) is a micronutrient that is essential for plant life and development [17]; nevertheless, its availability in soils is often limited [40,41] and variable in both space and time [1]. Therefore, distinct regions of the same root system might experience different nutrient provisions and this condition could possibly induce a reciprocal communication within the plant.

Few studies have been carried out to dissect the effects of a heterogeneous Fe provision in dicot plants, showing that IRT1 and FRO2 are controlled at both local and systemic level [23]. De Nisi et al. (2012) applied a split-root approach proposing that Fe itself might represent the local signal for the induction of the uptake system, whilst the systemic regulation was ascribed either to the lack of Fe or to a long distance signal, as for instance plant hormones [25]. The data hereby conveyed confirm that, in Fe deficient plants, the resupply with Fe stimulated the increase in the FRO enzymatic activity, required for the micronutrient uptake (Figure 1). Interestingly, the same behavior was observed in both non-split and split plants as already showed by De Nisi et al. (2012).

In order to further understand the possible role of Fe as regulator of its uptake and homeostasis, an approach based on the resupply of split plants with labelled Fe (^{57}Fe) was adopted. Indeed, it has been hypothesized that Fe bioavailability plays both a positive role in the local Fe deficiency response and a negative regulation role in the systemic signaling [42]. Following the resupply, a strong accumulation of ^{57}Fe was observed in the treated root part already after 12 h (Figure 2A); this phenomenon could be ascribed to the upregulated biochemical and molecular activities observed in the resupplied root portions (Figure 1A; De Nisi et al., 2012; Vert et al., 2003), consistent with the positive role of Fe supply in the local regulation of the Fe deficiency response (Gayomba et al., 2015). At the same time point, the accumulation of ^{57}Fe has been also observed in the non-resupplied root portion and in the shoot tissue (Figure 1A and C) as compared to (-/-) plants; these results suggested a quick distribution of Fe from the resupplied root portion to both leaves and non-resupplied root portion. Consistently, the analyses of images obtained through the real-time positron-emitting tracer ^{52}Fe demonstrated that, once taken up, Fe reached, through the xylem, the discrimination center (DC) located at the basal part of the shoots; at DC, the translocation from xylem to phloem could also occur [43]. From the DC, Fe is preferentially translocated to the younger leaves; however, it is also assumed that it might be transported via phloem to developing tissues, featuring Fe deficiency condition [25], that, in this case, might be also represented by the non-resupplied portion of the root system. At the following time points (24 and 48 h), the ^{57}Fe concentration in the resupplied portion of the root system reached a plateau (Figure 2A), whilst it constantly increased in the leaf tissue (Figure 2C and Figure 3). Nevertheless, no pronounced difference was highlighted in distinct areas of the mesophyll analyzed (Figure 4), thus demonstrating a rather uniform distribution of the micronutrient.

316 Interestingly, in the non-resupplied portion of the root system, the ^{57}Fe concentration did not show
317 any alteration between 12 and 24 hours, whilst it increased afterwards (Figure 1A). These data might
318 demonstrate that, immediately after the resupply, plants tend to distribute Fe from the DC to the whole
319 plant [25,43], most likely with the aim of alleviating the Fe deficiency response. According to the
320 data already available in the literature, the long distance signaling about Fe status in plants is mediated
321 by the micronutrient concentration in the phloem [44–47], that is involved in repressing the Fe
322 deficiency response in roots. Nonetheless, Figure 1A and C also showed that, within 24 h, Fe is
323 preferentially accumulated in the leaves, rather than in the non-resupplied root portion, most likely to
324 overcome a minimum threshold of Fe concentration that would allow shutting down the Fe deficiency
325 responses. In fact, it was observed that, in durum wheat plants, a Fe concentration of 35 µg g^{-1} DW
326 and 650 µg g^{-1} DW in shoot and root, respectively, is sufficient to prevent the rearrangement of S
327 metabolism required to sustain an efficient Fe deficiency response [48]. The activation of the Fe
328 translocation process from the resupplied roots towards the leaves is further supported by the
329 progressive decrease in the ^{56}Fe concentration in the resupplied root portion (Figure 1B) and by the
330 slight trend to the increase observed in the leaf tissue over the time (Figure 1D and Figure 3), that is
331 in good agreement with the Fe translocation models already proposed [25].
332 The Fe starvation is very well known to induce an alteration in the uptake and accumulation of other
333 mineral element in plants, causing unbalances in the ionomic profile of tissues [49–52]. In particular,
334 Fe starved plants feature higher concentrations of divalent cations, e.g. Cu^{2+}, Mn^{2+}, Zn^{2+} [50–52],
335 since these latter can be also transported by the Fe transporter IRT1 [53,54]. Interestingly, the data
336 presented demonstrated that, in the resupplied plants, Zn underwent a redistribution over the time at
337 leaf level, most likely competing with the scarcely available Fe (Figure 5A). Indeed, the binding
338 selectivity and specificity of metals to a given ligand is determined by several properties, such as the
339 affinity and the concentration of the metal in the cytosol [55,56]. According to the Irving-Williams
340 series, Zn^{2+} has a higher affinity for the organic ligands, such as nicotianamine (NA), as compared to
341 Fe^{2+} [57,58]. Nonetheless, at physiological conditions, Fe concentration in the cytosol is several order
342 of magnitude higher as compared to Zn [59], thus explaining why NA is preferentially associated
343 with Fe^{2+}rather than with Zn^{2+}. Therefore, both the low specificity of IRT1 transporters [53] and the
344 increased concentration in the apoplast, resulting in a higher chelation with organic ligands [57,59],
345 can contribute to the higher uptake of Zn at root level [50–52] and to the subsequent microelement
346 redistribution observed at leaf level.
347 In conclusion, our data suggest that in field conditions, where roots are exposed to heterogeneous
348 distribution of Fe, the micronutrient is taken up by the root tract in contact with the “Fe patches” and
349 then distributed to sink organs displaying Fe deficiency symptoms (Figure 6). Yet, after a small

increase in the Fe concentration showed by the Fe starved root portion, the micronutrient is preferentially translocated towards leaves and only afterwards the root system is completely resupplied (Figure 6). These observations highlight a root-to-shoot-to-root Fe translocation route in cucumber plants grown on a patchy nutrient substrate. Indeed, the thorough understanding of the physiological processes underlying the Fe distribution in agricultural plants, grown in field conditions and experiencing an uneven availability of nutrients, will be of paramount importance in order i) to efficiently tune the application of fertilizers, especially in a vision of sustainable-oriented smart agriculture, and ii) to better exploit the resources already available in the agricultural soils.

Acknowledgments

The research was supported by grants from the Free University of Bolzano (TN2071).

Authors' contribution.

Designing of the research: SC, TM, RT

Performance of the research: FV, YP, RT, CP, MCF, GMB

Data analyses, collection, or interpretation: FV, YP, RT, CP, MCF, GMB, SA, TM, SC

Writing the manuscript: YP, RT, SA, TM, SC

References

[1] A. Hodge, The plastic plant: root responses to heterogeneous supplies of nutrients, New Phytol. 162 (2004) 9–24. https://doi.org/10.1111/j.1469-8137.2004.01015.x.

[2] M.L. Cain, S. Subler, J.P. Evans, M.-J. Fortin, Sampling spatial and temporal variation in soil nitrogen availability, Oecologia. 118 (1999) 397–404. https://doi.org/10.1007/s004420050741.

[3] R.A. Farley, A.H. Fitter, The responses of seven co-occurring woodland herbaceous perennials to localized nutrient-rich patches, J. Ecol. 87 (1999) 849–859. https://doi.org/10.2307/2648641.

[4] M. Hendriks, E.J.W. Visser, I.G.S. Visschers, B.H.J. Aarts, H. de Caluwe, A.E. Smit-Tiekstra, W.H. van der Putten, H. de Kroon, L. Mommer, Root responses of grassland species to spatial heterogeneity of plant-soil feedback, Funct. Ecol. 29 (2015) 177–186. https://doi.org/10.1111/1365-2435.12367.

[5] M.C. Drew, L.R. Saker, Nutrient supply and the growth of the seminal root system in barley: II. Localized, compensatory increases in lateral root growth and rates op nitrate uptake when nitrate supply is restricted to only part of the root system, J. Exp. Bot. 26 (1975) 79–90. https://doi.org/10.1093/jxb/26.1.79.

[6] R.B. Jackson, M.M. Caldwell, The timing and degree of root proliferation in fertile-soil microsites for three cold-desert perennials, Oecologia. 81 (1989) 149–153. https://doi.org/10.1007/BF00379798.

[7] C.J. Bilbrough, M.M. Caldwell, The effects of shading and N status on root proliferation in nutrient patches by the perennial grass Agropyron desertorum in the field, Oecologia. 103 (1995) 10–16. https://doi.org/10.1007/BF00328419.

[8] H. Zhang, B.G. Forde, Regulation of Arabidopsis root development by nitrate availability., J. Exp. Bot. 51 (2000) 51–9. http://www.ncbi.nlm.nih.gov/pubmed/10938795 (accessed March 4, 2019).

[9] H. Zhang, A. Jennings, P.W. Barlow, B.G. Forde, Dual pathways for regulation of root branching by nitrate., Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 6529–34. http://www.ncbi.nlm.nih.gov/pubmed/10339622 (accessed March 4, 2019).

[10] M.M.I. van Vuuren, D. Robinson, B.S. Griffiths, Nutrient inflow and root proliferation during the exploitation of a temporally and spatially discrete source of nitrogen in soil, Plant Soil. 178 (1996) 185–192. https://doi.org/10.1007/BF00011582.

[11] A. Hodge, D. Robinson, B.S. Griffiths, A.H. Fitter, Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete, Plant,

Cell Environ. 22 (1999) 811–820. https://doi.org/10.1046/j.1365-3040.1999.00454.x.
[12] A. Hodge, J. Stewart, D. Robinson, B.. Griffiths, A.. Fitter, Plant N capture and microfaunal dynamics from decomposing grass and earthworm residues in soil, Soil Biol. Biochem. 32 (2000) 1763–1772. https://doi.org/10.1016/S0038-0717(00)00095-X.
[13] A. Larigauderie, J.H. Richards, Root proliferation characteristics of seven perennial arid-land grasses in nutrient-enriched microsites, Oecologia. 99 (1994) 102–111. https://doi.org/10.1007/BF00317089.
[14] I.G. Burns, Short- and long-term effects of a change in the spatial distribution of nitrate in the root zone on N uptake, growth and root development of young lettuce plants, Plant. Cell Environ. 14 (1991) 21–33. https://doi.org/10.1111/j.1365-3040.1991.tb01368.x.
[15] D. Robinson, The responses of plants to non-uniform supplies of nutrients, New Phytol. 127 (1994) 635–674. https://doi.org/10.1111/j.1469-8137.1994.tb02969.x.
[16] M.C. Drew, L.R. Saker, Nutrient supply and the growth of the seminal root system in barley: III. Compensatory increases in growth of lateral roots, and in rates of phosphate uptake, in response to a localized supply of phosphate, J. Exp. Bot. 29 (1978) 435–451. https://doi.org/10.1093/jxb/29.2.435.
[17] P. Marschner, Marschner's mineral nutrition of higher plants, 3rd ed., London, 2012.
[18] J. Balk, T.A. Schaedler, Iron cofactor assembly in plants, Annu. Rev. Plant Biol. 65 (2014) 125–153. https://doi.org/10.1146/annurev-arplant-050213-035759.
[19] J.-F. Briat, C. Dubos, F. Gaymard, Iron nutrition, biomass production, and plant product quality., Trends Plant Sci. 20 (2015) 33–40. https://doi.org/10.1016/j.tplants.2014.07.005.
[20] K. Hans Wedepohl, The composition of the continental crust, Geochim. Cosmochim. Acta. 59 (1995) 1217–1232. https://doi.org/10.1016/0016-7037(95)00038-2.
[21] M.L. Guerinot, Y. Yi, Iron: nutritious, noxious, and not readily available., Plant Physiol. 104 (1994) 815–820. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=160677&tool=pmcentrez&rendertype=abstract.
[22] T. Kobayashi, N.K. Nishizawa, Iron uptake, translocation, and regulation in higher plants., Annu. Rev. Plant Biol. 63 (2012) 131–52. https://doi.org/10.1146/annurev-arplant-042811-105522.
[23] G.A. Vert, J.-F. Briat, C. Curie, Dual regulation of the Arabidopsis high-affinity root Iron uptake system by local and long-distance signals, Plant Physiol. 132 (2003) 796 LP – 804. https://doi.org/10.1104/pp.102.016089.
[24] P. De Nisi, G. Vigani, M. Dell'Orto, G. Zocchi, Application of the split root technique to

437 study iron uptake in cucumber plants., Plant Physiol. Biochem. 57 (2012) 168–74.
438 https://doi.org/10.1016/j.plaphy.2012.05.022.
439 [25] T. Kobayashi, T. Nozoye, N.K. Nishizawa, Iron transport and its regulation in plants, Free
440 Radic. Biol. Med. 133 (2019) 11–20. https://doi.org/10.1016/j.freeradbiomed.2018.10.439.
441 [26] Y. Pii, L. Marastoni, C. Springeth, M.C.M.C. Fontanella, G.M.G.M. Beone, S. Cesco, T.
442 Mimmo, Modulation of Fe acquisition process by *Azospirillum brasilense* in cucumber
443 plants, Environ. Exp. Bot. 130 (2016) 216–225.
444 https://doi.org/10.1016/j.envexpbot.2016.06.011.
445 [27] E. Coppa, S. Celletti, Y. Pii, T. Mimmo, S. Cesco, S. Astolfi, Revisiting Fe/S interplay in
446 tomato: A split-root approach to study the systemic and local responses, Plant Sci. 276
447 (2018) 134–142. https://doi.org/10.1016/J.PLANTSCI.2018.08.015.
448 [28] G. Vizzotto, R. Pinton, C. Bomben, S. Cesco, Z. Varanini, G. Costa, Iron reduction in iron-
449 stressed plants of *Actinidia deliciosa* genotypes: Involvement of PM Fe(III)-chelate reductase
450 and H^+-ATPase activity, J. Plant Nutr. 22 (1999) 479–488.
451 http://www.scopus.com/inward/record.url?eid=2-s2.0-
452 0033035501&partnerID=40&md5=ffee0dd7b317f42c15532f478cdfb962.
453 [29] M.-R. Martínez-Cuenca, M.Á. Forner-Giner, D.J. Iglesias, E. Primo-Millo, F. Legaz,
454 Strategy I responses to Fe-deficiency of two *Citrus* rootstocks differing in their tolerance to
455 iron chlorosis, Sci. Hortic. (Amsterdam). 153 (2013) 56–63.
456 https://doi.org/10.1016/j.scienta.2013.01.009.
457 [30] C.J. Park, J.K. Suh, R.W. Lee, S.H. Lee, Determination of Cd, Cu, Zn and Pb in rice flour
458 reference materials by isotope dilution inductively coupled plasma mass spectrometry, Anal.
459 Sci. 13 (1997) 429–432. http://www.scopus.com/inward/record.url?eid=2-s2.0-
460 0141689960&partnerID=40&md5=40a5411bef282f318837ec2257b05a7a.
461 [31] C. Sariego Muniz, J. M. Marchante Gayon, J. Ignacio Garcia Alonso, A. Sanz-Medel,
462 Accurate determination of iron, copper and zinc in human serum by isotope dilution analysis
463 using double focusing ICP-MS, J. Anal. At. Spectrom. 14 (1999) 1505–1510.
464 https://doi.org/10.1039/A901751H.
465 [32] J. Wu, E.A. Boyle, Determination of iron in seawater by high-resolution isotope dilution
466 inductively coupled plasma mass spectrometry after $Mg(OH)_2$ coprecipitation, Anal. Chim.
467 Acta. 367 (1998) 183–191. https://doi.org/http://dx.doi.org/10.1016/S0003-2670(98)00145-7.
468 [33] J. Meija, J.A. Caruso, Deconvolution of isobaric interferences in mass spectra, J. Am. Soc.
469 Mass Spectrom. 15 (2004) 654–658.
470 https://doi.org/http://dx.doi.org/10.1016/j.jasms.2003.12.016.

[34] P. Rodríguez-González, A. Rodríguez-Cea, J.I. García Alonso, A. Sanz-Medel, Species-specific isotope dilution analysis and isotope pattern deconvolution for butyltin compounds metabolism investigations, Anal. Chem. 77 (2005) 7724–7734. https://doi.org/10.1021/ac051091r.

[35] J. Giner Martinez-Sierra, F. Moreno Sanz, P. Herrero Espilez, J.M. Marchante Gayon, J.I. Garcia Alonso, Biosynthesis of sulfur-34 labelled yeast and its characterisation by multicollector-ICP-MS, J. Anal. At. Spectrom. 22 (2007) 1105–1112. https://doi.org/10.1039/B706217F.

[36] V.A. Solé, E. Papillon, M. Cotte, P. Walter, J. Susini, A multiplatform code for the analysis of energy-dispersive X-ray fluorescence spectra, Spectrochim. Acta Part B At. Spectrosc. 62 (2007) 63–68. https://doi.org/10.1016/J.SAB.2006.12.002.

[37] M. Alfeld, K. Janssens, Strategies for processing mega-pixel X-ray fluorescence hyperspectral data: a case study on a version of Caravaggio's painting Supper at Emmaus, J. Anal. At. Spectrom. 30 (2015) 777–789. https://doi.org/10.1039/C4JA00387J.

[38] G. Vigani, S. Bohic, F. Faoro, B. Vekemans, L. Vincze, R. Terzano, Cellular fractionation and nanoscopic X-ray fluorescence imaging analyses reveal changes of zinc distribution in leaf cells of iron-deficient plants, Front. Plant Sci. 9 (2018). https://doi.org/10.3389/fpls.2018.01112.

[39] R. Terzano, M. Alfeld, K. Janssens, B. Vekemans, T. Schoonjans, L. Vincze, N. Tomasi, R. Pinton, S. Cesco, Spatially resolved (semi)quantitative determination of iron (Fe) in plants by means of synchrotron micro X-ray fluorescence, Anal. Bioanal. Chem. 405 (2013) 3341–3350. https://doi.org/10.1007/s00216-013-6768-6.

[40] T. Mimmo, D. Del Buono, R. Terzano, N. Tomasi, G. Vigani, C. Crecchio, R. Pinton, G. Zocchi, S. Cesco, Rhizospheric organic compounds in the soil-microorganism-plant system: their role in iron availability, Eur. J. Soil Sci. 65 (2014) 629–642. https://doi.org/10.1111/ejss.12158.

[41] C. Colombo, G. Palumbo, J.-Z. He, R. Pinton, S. Cesco, Review on iron availability in soil: interaction of Fe minerals, plants, and microbes, J. Soils Sediments. (2013) 1–11. https://doi.org/10.1007/s11368-013-0814-z.

[42] S.R. Gayomba, Z. Zhai, H.-I. Jung, O.K. Vatamaniuk, Local and systemic signaling of iron status and its interactions with homeostasis of other essential elements., Front. Plant Sci. 6 (2015) 716. https://doi.org/10.3389/fpls.2015.00716.

[43] T. Tsukamoto, H. Nakanishi, H. Uchida, S. Watanabe, S. Matsuhashi, S. Mori, N.K. Nishizawa, ^{52}Fe translocation in barley as monitored by a positron-emitting tracer imaging

505 system (PETIS): Evidence for the direct translocation of fe from roots to young leaves via
506 phloem, Plant Cell Physiol. 50 (2009) 48–57. https://doi.org/10.1093/pcp/pcn192.
507 [44] F.M. Maas, D.A. van de Wetering, M.L. van Beusichem, H.F. Bienfait, Characterization of
508 Phloem iron and its possible role in the regulation of fe-efficiency reactions., Plant Physiol.
509 87 (1988) 167–71. https://doi.org/10.1104/pp.87.1.167.
510 [45] M.G. Stacey, A. Patel, W.E. McClain, M. Mathieu, M. Remley, E.E. Rogers, W. Gassmann,
511 D.G. Blevins, G. Stacey, The arabidopsis AtOPT3 protein functions in metal homeostasis
512 and movement of iron to developing seeds, Plant Physiol. 146 (2008) 589–601.
513 https://doi.org/10.1104/pp.107.108183.
514 [46] D.G. Mendoza-Cózatl, Q. Xie, G.Z. Akmakjian, T.O. Jobe, A. Patel, M.G. Stacey, L. Song,
515 D.W. Demoin, S.S. Jurisson, G. Stacey, J.I. Schroeder, OPT3 is a component of the iron-
516 signaling network between leaves and roots and misregulation of OPT3 leads to an over-
517 accumulation of cadmium in seeds., Mol. Plant. 7 (2014) 1455–1469.
518 https://doi.org/10.1093/mp/ssu067.
519 [47] Z. Zhai, S.R. Gayomba, H. Il Jung, N.K. Vimalakumari, M. Piñeros, E. Craft, M.A. Rutzke,
520 J. Danku, B. Lahner, T. Punshon, M. Lou Guerinot, D.E. Salt, L. V. Kochian, O.K.
521 Vatamaniuk, OPT3 is a phloem-specific iron transporter that is essential for systemic iron
522 signaling and redistribution of iron and cadmium in arabidopsis, Plant Cell. 26 (2014) 2249–
523 2264. https://doi.org/10.1105/tpc.114.123737.
524 [48] S. Celletti, Y. Pii, T. Mimmo, S. Cesco, S. Astolfi, The characterization of the adaptive
525 responses of durum wheat to different Fe availability highlights an optimum Fe requirement
526 threshold, Plant Physiol. Biochem. 109 (2016) 300–307.
527 https://doi.org/10.1016/j.plaphy.2016.10.010.
528 [49] N. Tomasi, C. Rizzardo, R. Monte, S. Gottardi, N. Jelali, R. Terzano, B. Vekemans, M. de
529 Nobili, Z. Varanini, R. Pinton, S. Cesco, Micro-analytical, physiological and molecular
530 aspects of Fe acquisition in leaves of Fe-deficient tomato plants re-supplied with natural Fe-
531 complexes in nutrient solution, Plant Soil. 325 (2009) 25–38.
532 http://www.scopus.com/inward/record.url?eid=2-s2.0-
533 71249126520&partnerID=40&md5=d481f161b87b37457d930ef4793ef9ba.
534 [50] N. Tomasi, T. Mimmo, R. Terzano, M. Alfeld, K. Janssens, L. Zanin, R. Pinton, Z. Varanini,
535 S. Cesco, Nutrient accumulation in leaves of Fe-deficient cucumber plants treated with
536 natural Fe complexes, Biol. Fertil. Soils. 50 (2014) 973–982.
537 http://www.scopus.com/inward/record.url?eid=2-s2.0-
538 84905033437&partnerID=40&md5=0b77275fe0eaa85ad8cbb9f53e40fce4.

[51] Y. Pii, S. Cesco, T. Mimmo, Shoot ionome to predict the synergism and antagonism between nutrients as affected by substrate and physiological status, Plant Physiol. Biochem. 94 (2015) 48–56. https://doi.org/10.1016/j.plaphy.2015.05.002.

[52] I.R. Baxter, O. Vitek, B. Lahner, B. Muthukumar, M. Borghi, J. Morrissey, M. Lou Guerinot, D.E. Salt, The leaf ionome as a multivariable system to detect a plant's physiological status., Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 12081–6. https://doi.org/10.1073/pnas.0804175105.

[53] Y. Korshunova, D. Eide, W. Gregg Clark, M. Lou Guerinot, H. Pakrasi, The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range, Plant Mol. Biol. 40 (1999) 37–44. https://doi.org/10.1023/A:1026438615520.

[54] E.L. Connolly, N.H. Campbell, N. Grotz, C.L. Prichard, M. Lou Guerinot, Overexpression of the *FRO2 Ferric Chelate Reductase* confers tolerance to growth on low iron and uncovers posttranscriptional control, Plant Physiol. 133 (2003) 1102–1110. https://doi.org/10.1104/pp.103.025122.

[55] D.P. Giedroc, A.I. Arunkumar, Metal sensor proteins: nature's metalloregulated allosteric switches, Dalt. Trans. (2007) 3107. https://doi.org/10.1039/b706769k.

[56] K.J. Waldron, J.C. Rutherford, D. Ford, N.J. Robinson, Metalloproteins and metal sensing, Nature. 460 (2009) 823–830. https://doi.org/10.1038/nature08300.

[57] H. Irving, R.J.P. Williams, The stability of transition-metal complexes, J. Chem. Soc. 3 (1953) 3192–3210. https://doi.org/10.1039/jr9530003192.

[58] C. Curie, G. Cassin, D. Couch, F. Divol, K. Higuchi, M. Le Jean, J. Misson, A. Schikora, P. Czernic, S. Mari, Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters, Ann. Bot. . 103 (2009) 1–11. https://doi.org/10.1093/aob/mcn207.

[59] C.E. Blaby-Haas, S.S. Merchant, Lysosome-related organelles as mediators of metal homeostasis., J. Biol. Chem. 289 (2014) 28129–36. https://doi.org/10.1074/jbc.R114.592618.

Figure Legends

Figure 1. Time course of reductase activity in cucumber split plants after Fe-EDTA resupply. A. Reductase activity detected in cucumber plants 12 hours after the resupply with Fe-EDTA. **B.** Reductase activity detected in cucumber plants 24 hours after the resupply with Fe-EDTA. **C.** Reductase activity detected in cucumber plants 48 hours after the resupply with Fe-EDTA. Data are presented as means ± SE (n = 3). Statistical significance has been tested by one-way ANOVA analysis with Tukey *post hoc* tests ($P<0.001$). Different letters indicate statistically different values.

Figure 2. Labelled (^{57}Fe) and natural (^{56}Fe) Fe quantification in cucumber split plants after Fe-EDTA resupply. A. ^{57}Fe quantification in the root tissue of split cucumber plants at 12, 24 and 48 h after the resupply. The resupply has been carried out only in one compartment corresponding to the closed bars. Data are presented as means ± SE (n = 3). Statistical significance has been tested by one-way ANOVA analysis with Tukey *post hoc* tests ($P<0.001$). Different letters indicate statistically different values. Small letters indicate significance among different time points for resupplied plants. Capital letters indicate significance within the same treatment among different time points for not-resupplied plants. Within each time point, the statistical significance between resupplied and not-resupplied plants has been tested by Student's *t*-test. **B.** ^{56}Fe quantification in the root tissue of split cucumber plants at 12, 24 and 48 h after the resupply. The resupply has been carried out only in one compartment corresponding to the closed bars. Data are presented as means ± SE (n = 3). Statistical significance has been tested by one-way ANOVA analysis with Tukey *post hoc* tests ($P<0.001$). Different letters indicate statistically different values. Small letters indicate significance among different time points for resupplied plants. Capital letters indicate significance within the same treatment among different time points for not-resupplied plants. Within each time point, the statistical significance between resupplied and not-resupplied plants has been tested by Student's *t*-test. **C.** ^{57}Fe quantification in the leaves of split cucumber plants at 12, 24 and 48 h after the resupply. Data are presented as means ± SE (n = 3). Statistical significance has been tested by one-way ANOVA analysis with Tukey *post hoc* tests ($P<0.001$). Different letters indicate statistically different values. **D.** ^{56}Fe quantification in the leaves of split cucumber plants at 12, 24 and 48 h after the resupply. Data are presented as means ± SE (n = 3). Statistical significance has been tested by one-way ANOVA analysis with Tukey *post hoc* tests ($P<0.001$).

Figure 3. µXRF analyses of cucumber leaves. A. Representative µ-XRF distribution maps for Fe cucumber leaves. Brighter colours correspond to higher element concentrations. **B.** Quantification of total Fe concentration in leaves obtained by portable XRF instrument. Data are presented as means ± SE (n = 3). Statistical significance has been tested by one-way ANOVA analysis with Tukey *post hoc* tests (P<0.001). Different letters indicate statistically different values. Small letters indicate significance among different treatments within the single time point. Capital letters indicate significance within the same treatment among the different time points.

Figure 4. Uniform Fe distribution in the leaves of Fe-resupplied split cucumber plants. Fe relative concentrations calculated in 8 different leaf regions (as shown in the image below) from µ-XRF data (greyscale image) for a split cucumber plant (-/+) after 12 h from Fe resupply.

Figure 5. Analyses of Zn and Ca distribution in cucumber leaves. A. Representative µ-XRF distribution maps for Zn in cucumber leaves. **B.** Representative µ-XRF distribution maps for Ca in cucumber leaves. Brighter colours correspond to higher element concentrations.

Figure 6. Schematic representation of the Fe translocation model. Iron is taken up by roots grown in Fe rich patches of the soil and it is translocated via xylem (**1**) to the discrimination centre (DC) located at the base of the aerial part of the plant. At DC level, the translocation of Fe from xylem to phloem can occur. From DC, Fe is preferentially distributed to leaves (**2**) and, to a lower extent, to roots growing in Fe deficient soil patches (**2**). After the complete resupply of leaves, Fe is distributed to the Fe deficient root tracts, either from the shoots, from the DC or both (**3**), to overcome the micronutrient concentration threshold necessary to turn off the Fe deficiency responses.

Supplementary Figure 1. Schematic drawing of the experimental set up.

Supplementary Figure 2: A. Representative µ-XRF distribution maps for Mn in cucumber leaves. B. Representative µ-XRF distribution maps for Cu in cucumber leaves. Brighter colours correspond to higher element concentrations.

ha formattato: Evidenziato

ha formattato: Evidenziato

ha formattato: Evidenziato

ha formattato: Evidenziato

Supplementary Table 1. Instrumental operating conditions of ICP MS with octopole reaction cell.

Instrumental settings	
Rf power (W)	1550
Smpl Depth (mm)	5.6
Carrier Gas (L min^{-1})	0.7
Makeup Gas (L min^{-1})	0.38
Sampler and skimmer cone	Ni
Collision/Reaction cell parameters	
He gas flow (mL min^{-1})	4.4
OctP Bias (V)	-20
OctP RF (V)	190
Energy Discriminator (V)	5
Data Acquisition Parameters	
Integration time/mass (sec)	3
Points per peak	3
Monitored isotopes	54, 56, 57, 58