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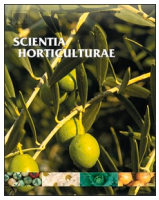

Iron nutrition in agriculture: From synthetic chelates to biochelates

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ABSTRACT

Iron (Fe) is an essential micronutrient for plant growth and productivity and, among other micronutrients, is the one required in higher amounts. In most soils, Fe occurs at relatively high concentrations; nevertheless, in calcareous soils, Fe bioavailability can be very limited, thus leading plants to develop Fe deficiency symptoms. At present, Fe fertilization still represents the most frequent approach adopted in agriculture to prevent or remediate Fe chlorosis. For soil applications, Fe fertilizers are based on Fe chelated with aminocarboxylate synthetic ligands (e.g. HEDTA, EDTA, DTPA, EDDHA), which are effective in maintaining Fe in the soil solution, even in alkaline soils, and thus in increasing its bioavailability for plant uptake. Nevertheless, Fe-aminocarboxylate complexes presents some limitations, related for instance to pH-dependent effectiveness, stability, persistence in the environment and ligand exchange reactions. In this context, vegetal-derived protein hydrolysates might represent good sources of biochelating ligands for developing innovative Fe fertilizers for sustainable agriculture. Protein hydrolysates are composed by small peptides, which, on one hand, can chelate Fe via amino acid side chains, carboxylate groups of C-termini, amine groups of N-termini and N atoms of amide groups of the peptide backbone, whilst, on the other hand, small peptides can also play a signaling role, triggering the acquisition of nutrient and morphogenetic processes in plants. The present paper reviews the current state of knowledge on traditional Fe fertilizers and, on the other side, explores the possible advantages in the application of biochelates as innovative Fe fertilizers. To further corroborate the hypothesis, three experimental trials have been carried out on three horticultural crops (cucumber, tomato, and strawberry) using a Fe-biochelate as Fe source and comparing it with the widely used traditional synthetic chelate Fe-EDDHA. The results in the three crops clearly show that Fe-biochelate is, at least, as efficient as Fe-EDDHA as source of micronutrient, even under circumneutral (pH 6.0) and alkaline conditions (pH 8.0), thus proving that Fe-biochelates can be promising alternatives to synthetic Fe chelates for the Fe nutrition management of crops. Considering the potential drawbacks of synthetic chelates (e.g., leachability, persistence, remobilization of toxic metals in soil), these findings might contribute enhancing the agriculture sustainability. In addition, Fe-biochelate could also find an application in soilless cultivation systems as an alternative to synthetic Fe chelates for Fe enrichment of edible plant tissues (biofortification), increasing their nutritional value.

1. Introduction

1.1. Importance of iron and limiting factors in plant uptake

Iron (Fe) is an essential micronutrients for plant growth and productivity and, among other micronutrients, is the one required in higher amounts (Kobayashi and Nishizawa, 2012). The essentiality of Fe is mainly due to its chemical properties, making it suitable for redox reactions and allowing it to play fundamental roles in biological processes, like photosynthesis, respiration, chlorophyll biosynthesis (Marschner, 2012). To acquire Fe from rhizosphere, plants have developed two mechanisms, Strategy I and Strategy II, also known as reducing and chelating strategies, respectively (Römheld and Marschner, 1986). In Strategy I plants (i.e., dicots and non-graminaceous monocots), the Fe^{3+}

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present in the rhizosphere is reduced to Fe^{2+} by the activity of the plasma membrane-bound enzyme Ferric Reductase Oxidase (FRO) (Robinson et al., 1999) and, afterwards, it is taken up by the Iron Regulated Transporter 1 (IRT1) (Eide et al., 1996; Varotto et al., 2002). In addition, prior reduction and uptake, plasma membrane H^+-ATPases are activated to extrude protons aimed at lowering rhizosphere pH and thus favoring the solubilization of sparingly soluble Fe^{3+} sources (Kobayashi and Nishizawa, 2012; Santi and Schmidt, 2009). When the Fe availability is limited, the three activities (FeIII-reduction, Fe^{2+}uptake, H^+ extrusion) are enhanced as an attempt to cope with the nutritional disorder (Kobayashi and Nishizawa, 2012). Differently, Strategy II plants (i.e., grasses) rely on the release of phytosiderophores (PSs), non-proteinogenic amino acids displaying a high affinity for Fe, for the acquisition of the micronutrient from the rhizosphere (Higuchi et al., 1999). In grasses, PSs release is mediated by the transporter Transporter of Mugineic acid family phytosiderophores (TOM1), which is located on the plasma membrane of root cells (Nozoye et al., 2011); once in the rhizosphere, PS chelate Fe^{3+} and, afterwards, the complex Fe^{3+}-PSs is imported in the root cell via the oligopeptide transporters Yellow Stripe 1 (YS1) and its orthologs YSL (Yellow Stripe Like) (Curie et al., 2001; Inoue et al., 2009). Nevertheless, recent pieces of evidence highlighted that the distinction between the two strategies might be not so clear and defined. Indeed, Strategy I plants have been shown to release exudates, like organic acids, phenolic compounds and flavonoids, which can have a Fe chelating function in the rhizosphere compartment (Cesco et al., 2010; Mimmo et al., 2014). More recently, Astolfi et al. (Astolfi et al., 2020) demonstrated, that under variable Fe provision in the growth medium, tomato plants were able to release 3-hydroxymugineic acid, an organic compound belonging to the class of PSs, and to modulate the expression of *YSL* genes. These observations further show that the two strategies might not be mutually exclusive, as already suggested for *Arachis hypogea* (Xiong et al., 2013) and demonstrated in rice (Kobayashi and Nishizawa, 2012).

In the majority of soils, Fe occurs in two oxidation states (Fe^{2+} and Fe^{3+}) at relatively high concentrations, ranging from 20 to 40 mg kg^{-1} (Colombo et al., 2013; Mimmo et al., 2014); it can be found in different primary and secondary minerals (e.g. olivine, biotite, vermiculite), albeit the most abundant Fe form is represented by (hydr)oxides, which are very little soluble in the soil solution (Colombo et al., 2013; Mimmo et al., 2014). Indeed, the solubility of Fe (hydr)oxides in soils depends on two main factors, pH and redox potential (Eh); neutral to alkaline pH values favor the precipitation of insoluble Fe forms, whereas acidic and reducing conditions promote the solubilization of Fe (Colombo et al., 2013). Considering that about 30% of the world's cultivated soils are calcareous (Marschner, 2012), Fe bioavailability can be very limited to plants; it has been estimated that, within the pH interval between 5.0 and 8.5, the Fe concentration in the soil solution ranges from 0.1 to 10^{-3} μM (Kraemer et al., 2006), whereas the average Fe concentration required for an optimal plant growth is 4 to 5 order of magnitude higher (Lemanceau et al., 2009). As a consequence of this discrepancy, plants often develop Fe deficiency symptoms, which include, for instance, the interveinal chlorosis of young leaves, a decrease in the photosynthesis rate and a reduced growth (Guerinot and Yi, 1994). To overcome Fe shortage, plants induce both morphological and molecular adaptations, as for instance by modifying their root system architecture and by triggering the expression of genes involved in Fe solubilization and acquisition from rhizosphere compartment (Li et al., 2016; Zhang et al., 2019). Additionally, several pieces of evidence have demonstrated that the role of rhizosphere microorganisms can also be important to help plant coping with suboptimal Fe concentrations in the growth medium (Pii et al., 2016, 2015a, 2015b).

1.2. Traditional fertilizer sources: pro and cons of synthetic chelates

Different strategies can be exploited to remediate Fe deficiency in plants; for instance, plants with a higher Fe efficiency might be adopted, or soil conditioners can be applied to improve Fe dissolution and root growth. Nevertheless, the use of Fe-based fertilizers still represent the most frequent and economically sustainable approach adopted in agriculture, applied either to the canopy, as foliar spray, or to the soil (Lucena, 2006). The soil application of inorganic Fe salts (e.g., $FeSO_4$) does not represent an efficient practice, since, depending on the soil chemical and physical characteristics, Fe can rapidly precipitate as Fe (hydr)oxide, thus resulting not available for plant nutrition (Lucena, 2006). As an alternative, Fe can be supplied in a chelated form with aminocarboxylate synthetic ligands, like HEDTA, EDTA, DTPA and EDDHA, which can be useful in maintaining Fe in the soil solution and thus in increasing plant ability to acquire the micronutrient from the rhizosphere compartment. Based on this feature, synthetic chelates also allow reducing the amount of fertilizer applied to crops (Lucena, 2006), thereby having an economic benefit. However, the stability of Fe complexes is strongly influenced by soil pH; only the most stable chelate (i.e., o,o-EDDHA/Fe^{3+}), and yet the most expensive, is able to maintain Fe in the soil solution, and transport it to the plant root, in highly calcareous soils (Lucena, 2006). On the other hand, in soilless conditions, pH adjustment of nutrient solutions and media is relatively easier than in soils (Tomasi et al., 2015), allowing for easier control and management of the pH values. In a context like this, it is preferable the use of more inexpensive and less stable Fe chelates (Sambo et al., 2019). It is also worth mentioning that Fe can also be taken up by plants as a complex with the aminocarboxylate ligands; these synthetic compounds were shown to have a good persistence in the plant tissues, to reduce plants resistance against pathogens and to affect the nutraceutical quality of agricultural products (Bienfait et al., 2004). Moreover, it must be considered that these synthetic Fe complexes are also poorly retained in the soil with high risk of synthetic chelates leaching (Cesco et al., 2000) with the environmental implications that this entails.

Another important aspect to be considered when using Fe chelates for the fertilization of crops is the stability of the complex in soils. As mentioned above, pH plays a crucial role; however, also the concentration of other cations can influence the speciation of complexes in the soil (Lucena, 2006). Indeed, this is determined by their stability constants, even though these parameters can have only a limited significance in the speciation prediction, when natural environments like calcareous soils are considered (Nowack, 2002). In fact, the metal ion capturing the greatest amount of chelating agent at the chemical equilibrium is determined by the product between the stability constant and the concentration of the free metal ion (Nowack, 2002). In the case of Fe^{3+}-EDTA ($\log K = 27.2$) (Ahrland et al., 1990), the concentration of free Fe^{3+} at pH above 7 is several orders of magnitude (about 6) lower than that of Ca^{2+}, which, having a strong affinity for EDTA, can efficiently outcompete Fe^{3+} to bind the chelating agent, causing the precipitation of Fe as (hydr)oxides (López-Rayo et al., 2019; Nowack, 2002). A similar antagonism has been also described between Fe and Zn (López-Rayo et al., 2019; Nowack, 2002), further demonstrating the limited applicability of less stable complexes for field fertilization approaches. More recently, it was observed that the concentration of *meso* o,o-FeEDDHA in the soil solution exponentially declined (Hernández-Apaolaza and Lucena, 2011; Schenkeveld et al., 2010, 2007); this phenomenon was shown to be independent from biotic factors (*i.e.*, plant uptake and microbial degradation) (Schenkeveld et al., 2012), whilst it was rather ascribed, as mentioned above, to cations displacement reactions (Nowack, 2002; Schenkeveld et al., 2010), with copper (Cu) as possible candidate, considering its great affinity for o,o-EDDHA isomers (Bannochie and Martell, 1989; Yunta et al., 2003b, 2003a). In general, the concentration of Cu in soils is sufficiently high to threaten the effectiveness of treatments based on FeEDDHA, i.e. the most stable Fe chelate. This context is further exacerbated by the increasing concentration of Cu in the agricultural soils, due to the extensive use of this metals as fungicides also in the organic farming systems (Cesco et al., 2021). Moreover, from the general point of view, the intricate chemical, biological and physical interactions occurring at the rhizosphere level

shaping nutrients availability and plant uptake make the soil-plant system even more complex and difficult to predictively manage (Terzano et al., 2015).

In spite of their use in agriculture, the environmental fate of aminocarboxylate synthetic ligands is attracting the attention in the last decades. It has been clearly demonstrated that some of the chelating compounds adopted (e.g. EDTA) are little biodegradable in soils and are therefore very persistent in natural systems (Tandy et al., 2004). This long persistence in the environments, together with their ability to alter the natural speciation of metals in soils and aquifers, can lead to an increased bioavailability of metals, both beneficial and toxic ones (Wenzel et al., 2003; Wu et al., 2004). These concerns are further corroborated by the application of chelating compounds in the remediation and phytoextraction approaches of soils contaminated with heavy metals (Sinegani et al., 2015). In these approaches, chelating agents are used to increase the availability of heavy metals, so that plants can extract them from contaminated soil to a higher efficiency (Sinegani et al., 2015). Nevertheless, different chelating compounds display a diverse phytoextraction efficiency; a study carried out on lead (Pb) contaminated soils showed that EDTA was the most efficient one in determining Pb mobilization from the soil particles, followed by HEDTA>DTPA>EGTA>EDDHA (Huang et al., 1997). However, these further observations indeed highlight that the accumulation of chelating compounds in agricultural soils, originated from fertilization strategies, could provoke the undesired mobilization of heavy metals that can enter the food chain, thereby affecting both the quality and the safety of agricultural products.

1.3. Plant biostimulants as innovative tool for enhancing iron nutrition of plants

Plants biostimulants (PBs) are defined as a class of substances able to improve crop productivity and quality, increasing the availability of nutrients in the soil, ameliorating nutrient use efficiency of plants, and promoting the degradation and humification of organic substances in soils (Rouphael and Colla, 2020). Overall, PBs feature a variegate nature thereby including a broad spectrum of substances, all of these exerting the above-mentioned beneficial effects on plants, albeit their precise mode of action is still elusive (du Jardin, 2015). Among other PBs, in the last years, beneficial microorganisms, humic substances and protein hydrolysates are attracting great attention as a possible greener alternative respect to traditional fertilizers for managing Fe nutrition in crop plants (Celletti et al., 2020; Pii et al., 2015a; Zanin et al., 2019). Beneficial microorganisms, also known as Plant Growth-Promoting Rhizobacteria (PGPR), are soil microbiota, which are able to establish a profitable relationship with plants by colonizing their rhizosphere and by enhancing plant growth (Pii et al., 2015a). The growth promotion effect can be achieved through different mechanisms, as for instance organic matter mineralization, biological control against soil-borne pathogens, biological nitrogen fixation, and the production of phytohormones-like molecules (Glick, 2012). A very interesting feature of PGPR is their ability to affect the biogeochemical cycles of elements, thereby modulating nutrients bioavailability for plants (Alegria Terrazas et al., 2016); several bacteria have been characterized for their phosphorus-solubilizing properties (Glick, 2012; Pii et al., 2015a), whilst other species have been demonstrated to enhance the availability of micronutrients (e.g. Fe) by releasing chelating compounds like microbial siderophores (Alegria Terrazas et al., 2016; Pii et al., 2015a). Nevertheless, several pieces of evidence highlight that root colonization by PGPR can modulate plant physiology, thereby affecting the efficiency of plants to acquire nutrients from the external medium (Pii et al., 2015a). In the specific case of Fe nutrition, for instance, the inoculation with *Azospirillum brasilense* caused in cucumber plants a modulation in the qualitative and quantitative root exudation profile (Pii et al., 2015b) and an upregulation of the mechanisms involved in Fe acquisition, even though plants were supplied with adequate micronutrient concentration (Pii et al., 2016). This indeed led to enhanced Fe uptake at root level (about 4 times higher) and to a greater Fe concentration in leaves, as compared to not inoculated plants (Pii et al., 2016, 2015b).

Arbuscular mycorrhizal fungi and endophytic fungi such as *Trichoderma atroviride* play also an important role in Fe acquisition of plants. Inoculation of cucumber plants with *Rhizoglomus irregulare* BEG72 (former *Glomus intraradices*) significantly increased Fe concentration in leaf and fruit tissues in comparison with uninoculated plants by 14 and 20%, respectively (Rouphael et al., 2010). Similar results were obtained on Fe concentration of corn and tomato plants inoculated with *Rhizoglomus irregulare* BEG72 and *Funneliformis mosseae* BEG234 (Saia et al., 2019). Moreover, Fe concentration of leaves in zucchini and lettuce plants was enhanced by root inoculation with a tablet containing a microbial consortium of *Rhizoglomus irregulare* BEG72 (former *Glomus intraradices* BEG72) and *Trichoderma atroviride* MUCL 45632 (Colla et al., 2015b). It is well-known that mycorrhizae can enhance the plant uptake of various mineral nutrients, including Fe, through an increase of surface area for nutrient absorption provided by the fungal mycelium. Similarly, *Trichoderma* spp. can increase surface area for nutrient absorption by stimulating fine root growth resulted from the fungal synthesis of indole-3-acetic acid (IAA) and IAA-related compounds (Colla et al., 2015b). Moreover, it has been demonstrated that arbuscular mycorrhizal fungi and *Trichoderma atroviride* can enhance Fe availability for plant uptake through biosynthesis of siderophores as chelating agents. For instance, the glomuferrin siderophore was isolated from *Tagetes patula* roots infected with arbuscular mycorrhizal fungi of the genus *Glomus* after 2–3 weeks of growth in pots containing low-Fe sand and irrigated with Hoagland solution (Winkelmann, 2017). Similarly, *Trichoderma atroviride* MUCL 45632 was able to produce under in vitro conditions two types of siderophores: hydroxamates and catechols (Colla et al., 2015b).

Humic substances (HS) represent the main pool of organic carbon in the soil (Canellas et al., 2015) and they are mainly originated from the partial degradation and re-synthesis of organic plant residues, through a polymerization/polycondensation of phenolic molecules, which are predominantly produced by microbial degradation of lignin (Zanin et al., 2019). The biostimulant activities of HS on plants have been widely documented in the literature (Canellas et al., 2015), occurring with significant increase in the accumulation of both root and shoot biomass, independently from the plant species considered (Rose et al., 2014). Specifically at root level, HS induce changes in the root system architecture and root growth dynamics, therefore resulting in an increased root size, branching and a higher density of root hairs (Canellas and Olivares, 2014; Pinton et al., 1999, 1997a), which are by some authors ascribed to an auxin-like effect on plants (Muscolo et al., 1998). Moreover, the HS stimulation at the root level of the plasma membrane H^+-ATPase activity, the key enzyme of the transmembrane electrochemical gradient underlying the movements of solutes across the membrane, has long been demonstrated (Pinton et al., 1997b). In addition, several studies have clearly demonstrated that HS can contribute to Fe nutrition of plants by forming soluble Fe-HS complexes, which can interact with plant uptake mechanisms as natural Fe-chelates (Zanin et al., 2019). Experiments carried out using a water-extractable humic fraction (WEHS) (Cesco et al., 2000) complexed to Fe (Fe-WEHS) demonstrated that both Strategy I and II plants could use this source to cope with Fe nutrition (Cesco et al., 2002; Pinton et al., 1999), even at pH values close to those found in calcareous soils (Cesco et al., 2002; Tomasi et al., 2013; Zamboni et al., 2016). Moreover, plants showed a higher Fe use efficiency (i.e., higher Fe accumulation and translocation) when supplied with Fe-WEHS as compared to other natural chelates, like Fe-citrate and Fe-PS (Tomasi et al., 2009, 2013; Zamboni et al., 2016); interestingly, the recovery observed in Fe-deficient plants resupplied with Fe-WEHS was coupled molecular (Tomasi et al., 2013; Zamboni et al., 2016) and biochemical (Pinton et al., 1999; Tomasi et al., 2013) modulation of the processes underpinning Fe acquisition strategies, unraveling the pivotal role played by

WEHS (Zamboni et al., 2016).

Besides HS, other biostimulant substances like protein hydrolysates (PHs) are able to enhance plant iron uptake (Celletti et al., 2020). PHs are a mixture of bioactive compounds as amino acids and peptides obtained from animal or vegetal protein sources through a process of enzymatic and/or thermal-chemical hydrolysis (Colla et al., 2015a). PHs may also contain carbohydrates, phenols, mineral elements, phytohormones and other organic compounds contributing to their biostimulant activity. PHs improve crop performances through direct and/or indirect effects: one of most significant direct effects is the increase of plant nutrition due to a higher nutrient acquisition process and metabolic use while the main indirect effect is the enhance of beneficial microorganisms at the rhizosphere level (Colla et al., 2017; Giordano et al., 2020; Rouphael et al., 2017). PHs can enhance plant nutrient uptake increasing bioavailability of nutrients in soil solution and promoting more root growth and active uptake process (Colla et al., 2015a). PHs-mediated improvement of nutrient bioavailability in soil solution has been associated to the conversion of mineral nutrients as inorganic ions to complexes that have higher solubility. The formation of mineral-organic complexes between mineral nutrients and various PHs-derived ligands such as peptides and amino acids has been reported for mineral cations including iron. The increase of iron bioavailability in soil solution can also result from the PHs-mediated enhancement of iron-solubilizing activity of microorganisms. For instance, Colla et al. (unpublished data) demonstrated that application of the vegetal-derived PH 'Trainer®' in the substrate at a rate of 2 mL L^{-1} enhanced the population of iron-solubilizing *Trichoderma atroviride* MUCL 45632 from 15 to 662 CFU g^{-1}. Vigorous root growth and long and dense root hairs are important crop traits for ensuring an efficient acquisition of nutrients (Wang et al., 2016). Several studies demonstrated that PHs can stimulate fast and early root proliferation increasing the root's absorptive surface area (Colla et al., 2015a); the above findings have been associated with the presence of bioactive compounds (e.g., signaling peptides such as 'root hair promoting peptide' and amino acids like 'glutamate') with auxin-like activity in PHs (Colla et al., 2014, 2015a). It is interesting to notice that aerial application of the vegetal-derived pH 'Trainer®' or its bioactive fraction on tomato cuttings was as effective as basal application in root growth stimulation indicating that signaling compounds such as small peptides were able to act locally and systemically (Lucini et al., 2020); metabolomic analysis of tomato tissues reveled that the PHs-mediated root growth stimulation resulted from a complex cross-talk among different phytohormones (auxins, cytokinins, abscisic acids, gibberellins, brassinosteroids) which coordinates morphological root responses to PH application (Lucini et al., 2020). Sestili et al. (2018) also reported that the foliar and especially substrate drench applications of the vegetal-derived PH 'Trainer®' on tomato plants stimulated root biomass accumulation; these findings were associated with the PH-mediated transcriptional downregulation of gene encoding for high-affinity nitrate transporter (*NRT2.1*) and repression of lateral root initiation (Little et al., 2005). In a hydroponic study on cucumber and tomato grown under Fe deficiency, Celletti et al. (2020) demonstrated that foliar applications of the vegetal-derived PH 'Trainer®' enhanced Fe concentration in shoots of both crops; however, the enhanced Fe concentration in the PH-treated shoots was associated with an increase of shoot and root biomass only in tomato crop indicating a genotype-dependent response to PH application. Similarly, Cerdán et al. (2013) reported that foliar and especially root applications of a vegetal-derived PH stimulated plant growth (shoots and roots) and enhanced Fe-nutritional status of tomato seedlings grown in quartz sand under lime-induced Fe deficiency. On the contrary, animal-derived PH caused a severe plant-growth depression without any improvement on Fe nutrition of tomato seedlings. The above findings were mainly attributed to the beneficial effects of glutamic acid, largely present in plant-derived product, on nitrogen metabolism and chlorophyll biosynthesis. Moreover, signaling peptides contained in PHs can positively regulates many Fe-deficiency-inducible genes for Fe uptake. For instance, a recent study (Grillet et al., 2018) reported that a ubiquitous peptide family (called IRON MAN) acts as a phloem-mobile signal to control Fe uptake and transport in plants.

1.4. Biochelates as innovative fertilizers combining biostimulants and nutrient metal elements

Plant nutrition can be improved by combining biostimulant effects and nutrients supply in advanced fertilizers like biochelates. A biochelate can be defined as an organic compound consisting of a central metal atom attached to one naturally occurring organic molecule, called ligand (e.g., peptide). Metal biochelates can be obtained with plant nutrients existing in cationic form (e.g., calcium, iron, manganese, zinc, copper). Metal biochelates with micronutrients are more bioavailable in the soil solution than the corresponding inorganic salts or oxides for plant uptake especially under alkaline conditions. Moreover, biochelates contain environmentally friendly chelating agents that are fully biodegradable and nontoxic for humans and animals, and therefore they can be used in agriculture without the health and environmental concerns raised for synthetic chelates, as discussed above. Despite the great potential of biochelates as fertilizers, this technology is still slightly used in agriculture. Biochelates, such as metal-peptide complexes, are currently largely adopted in food and feed industry to enhance mineral bioavailability for human or animal nutrition (Chaquilla-Quilca et al., 2016; Li et al., 2017). Peptides can be very effective, and often specific, biochelators for a variety of metal ions: they have many potential donor atoms through the peptide backbone and amino acid side chains. The metal-peptide complexes created occur in many arrangements depending on the amino acid sequence and number, and the pH environment. In a single amino acid, there are few donor groups (e.g., two or three) that can complex the metal like the carbonyl group and the N-terminal amino group. In the case of peptides, the potential donor atom is further expanded to other donor groups like the amide in the peptide backbone which create a significantly stronger metal binding. Coordination of metal ion begins at the N-terminal amino nitrogen of peptide. The adjacent carbonyl oxygen is the second donor to complete the chelate ring. By raising the pH, the metal ion can deprotonate successive peptide nitrogens forming metal-N- bonds; the number of peptide nitrogens involved in the metal bonding are enhanced by increasing the pH. On the contrary, the chelating capacity of peptides is strongly reduced by increasing the acidity of solution being very poor below pH 5; this behavior is due to the conversion of anionic chelating amino terminal ligands to cationic protonated forms with the increase of H^+ concentration in the solution (Ballarini and Predieri, 2009). A variety of metal-chelating peptides has been generated and identified from different food sources, such as milk, egg, soybean and sea cucumber (Sun et al., 2020). The mineral-chelating properties of peptides are attributed to the structural diversity of their backbone, which contains both the terminal carboxyl and amino groups, and the side chains of amino acid residues (Li et al., 2017). Small peptides have a higher proportion of amino and carboxyl groups (the oxygen of the C-terminus and the nitrogen of the N-terminus) resulting in higher Fe chelation activity, as reported for red seaweed peptides (Cian et al., 2016) and *Stichopus japonicus* derived PHs (Sun et al., 2017). However, specific amino acids affect stability and effectiveness of Fe-chelatable peptides. Peptides containing glutamic and aspartic acids have higher affinities for Fe^{3+} and peptides containing arginine and asparagine prefer to form chelates with Fe^{2+}. Histidine-, serine- and cysteine-rich peptides have higher Fe-chelating ability (Cian et al., 2016; Torres-Fuentes et al., 2012; Wang et al., 2012). According to Cruz-Huerta et al. (2016), Fe-bound peptides exhibited common structural characteristics, such as an abundance of aspartic acid, glutamic acid, and proline and these characteristics are common in vegetal-derived peptides. PHs can represent a valuable source of peptides having chelating properties with iron. Small peptides (< 5 kDa) have been identified from mung bean PHs for the Fe-binding properties due to the abundance of hydrophobic amino acid

residues such as proline, alanine, aspartic acid, isoleucine, leucine, and synergistic effects of the pyrrolidine ring, carboxyl, and alkyl group existing in proline, aspartic acid, and leucine, respectively (Budseekoad et al., 2018). In African yam bean seed-derived PHs, the amino acids glutammic acid, glutammine, aspartic acid, asparagine, glycine, leucine, lysine, alanine, and phenylalanine have been selected for Fe-binging ability (Ajibola et al., 2011). For the same Fe-binding ability, other amino acids have been selected in chickpea PHs (Torres-Fuentes et al., 2012), barley glutelin hydrolysate (Xia et al., 2012), soybean PHs (Lv et al., 2013; Zhang et al., 2012), sesame PHs (Wang et al., 2012). Moreover, Fe-peptide complexes can contain signaling peptides able to boost nutrient metal element uptake and utilization through the modulation of root morphology and transcription factors involved in iron homeostasis of plant tissues.

Recently, innovative fertilizers containing metal-biochelates were introduced in EU and US markets. These fertilizers contain a range of micronutrients (e.g., Fe, Zn, Mn) and calcium chelated with peptides obtained through enzymatic hydrolysis of vegetal-derived proteins. Preliminary studies showed these metal-biochelates have a good stability in the pH range 6–8 (Reynaud et al., unpublished data).

2. Iron biochelate as sustainable alternative to synthetic iron chelate: experimental evidences

Three agronomic trials were carried out in cucumber (Case Study 1), tomato (Case Study 2) and strawberry (Case Study 3) to compare the agronomic performances of a recently launched Fe-biochelate containing vegetal-derived peptides (KeyLan Fe - Hello Nature®, Anderson, IN 46,016, US) and the widely used syntetic Fe chelate (Sequestrene Life - Syngenta Italia S.p.A., 20,151 Milano, Italy). The tested Fe-biochelate (KeyLan Fe) contained 11% of water soluble Fe and 31% of vegetal-derived peptides. Sequestrene Life was a Fe-EDDHA chelate containing 7% of water soluble Fe in ferric form (Fe^{3+}). It is worth mentioning that KeyLan Fe contained ferrous iron (Fe^{2+}), which is physiologically more suitable for plant nutrition than Fe^{3+}. Both KeyLan Fe and Sequestrene Life were allowed in organic farming (Regulation (EC) No 889/2008).

2.1. Case study 1

The aim of Case Study 1 was to evaluate the effectiveness of the novel Fe-biochelate fertilizer in comparison with Fe-EDDHA in supplying Fe to hydroponic cucumber crop (*Cucumis sativus* L. - cv Chinese long) previously grown for 14 days under Fe-sufficiency (+Fe) or Fe-deficiency (-Fe) conditions, at two different pH values: 6 or 8. Fe-biochelate or Fe-EDDHA were applied for a period of 10 days. Cucumber plants were grown hydroponically in controlled conditions in a climatic growth chamber at the Free University of Bozen. The relative humidity was about 70% and the light intensity was 250 mmol m^{-2} s^{-1} with a day/night cycle of 14/10 h and 24°C/19°C. Cucumber seeds were first soaked overnight with 0.5 mM $CaSO_4$ and then germinated on a filter paper moistened with 0.5 mM $CaSO_4$ solution in darkness for 5 days (Nikolic et al., 2012). After the germination period, cucumber seedlings were transferred in pots containing continuously aerated nutrient solution (NS). The composition of NS was as follows (mM): 2 Ca $(NO_3)_2$, 0.7 K_2SO_4, 0.1 KH_2PO_4, 0.1 KCl, 0.5 $MgSO_4$, and (μM): 10 H_3BO_3, 0.5 $MnSO_4$, 0.2 $CuSO_4$, 0.1 $ZnSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$. Cucumber plants were grown either in Fe-sufficient (Fe was supplied as Fe(III)-DTPA, 80 μM) or in Fe-deficient conditions, as previously described (Pii et al., 2016). The pH of the solution was adjusted to either 6 with 1 M KOH or 8 by adding 10 mM $NaHCO_3$ and 5 mM $CaCO_3$. The NS was renewed every two days. After a growing period of 14 days, Fe-deficient plants were re-supplied with either Fe-EDDHA (Sequestrene Life - Syngenta Italia S.p.A., 20,151 Milano, Italy) or Fe-biochelate (KeyLan Fe - Hello Nature Inc, US), thereby adding Fe to a final concentration of 1 μM. Fe-supplied plants were further cultivated for 10 days. For limiting photochemical reduction phenomena of the micronutrient in the nutrient solution (Zancan et al., 2006) due to the Fe sources, beakers were covered with a black plastic foil during the entire experiment. Moreover, during this period, both the leaf SPAD index and the root ferric chelate reductase (FCR) activity were monitored at 0, 2, 4, 6, 8 and 10 days after treatments (DAT). At harvest, root system architecture (i.e., root length, root area, root diameter and root volume) was assessed through Winrhizo software (EPSON 1680, WinRHIZO Pro, 2003b; Regent Instruments Inc, Quebec, Canada); roots and shoots were separated, dried at 65°C until constant weight for the determination of root and shoot dry weight (RDW and SDW, respectively). All the assessments were carried out on three independent biological replicates. 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 (Vizzotto et al., 1999). Briefly, root systems were submerged in the reagent solution composed by 0.5 mM $CaSO_4$, 10 mM MES NaOH (pH 5.5), Fe(III)-EDTA 0.25 mM and BPDS 0.6 mM and incubated in the dark at 25°C. After 30 min incubation, the absorbance 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}.

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). To separate treatment means within each measured parameter, Tukey's multiple comparison test was performed at $P = 0.05$.

The monitoring of the leaf SPAD index during the resupply experiment clearly showed that, at pH 6, Fe-biochelate and Fe-EDDHA had the same effects on the chlorophyll content recovery (Fig. 1A); indeed, already at 2 DAT, Fe-biochelate treated plants showed an increasing trend in the leaf SPAD index, with respect to -Fe samples, albeit not statistically significant differences (Fig. 1A). At 4 DAT, on the other hand, the SPAD index of Fe-biochelate treated leaves was significantly higher as compared to negative controls, and completely equivalent to the values obtained with commercial Fe-EDDHA (Fig. 1A). The same SPAD pattern was observed until the end of the experiment with no significant differences between the Fe-biochelate and Fe-EDDHA treatments. Considering that alleviation of leaf chlorosis is related to an improved Fe nutrition, cucumber plants were also investigated for their ability to reduce Fe(III) to Fe(II) at root level, by monitoring the FCR activity during the resupply experiment. As shown in Fig. 1B, -Fe and +Fe plants presented a significant difference in the FCR activity, at all the time points considered, demonstrating that -Fe plants were continuously up-regulating this enzymatic activity as a response to Fe starvation. Interestingly, when -Fe plants were resupplied with either Fe-biochelate or Fe-EDDHA (i.e., from 2 DAT and onwards), FCR activity showed a significant decrease with respect to untreated plants (-Fe); the response of plants to the two different Fe sources was statistically not different and very close to the FCR activity displayed by +Fe plants (Fig. 1B). These observations suggested that Fe-biochelate were as efficient as Fe-EDDHA in providing starved plants with adequate Fe concentration to induce the down-regulation of the Fe deficiency response (Valentinuzzi et al., 2020). On the other hand, the results of analyses carried out on cucumber plants grown in a nutrient solution featuring a higher pH value (i.e., pH 8) showed a significantly different behavior compared to previous case (Fig. 1C and D). The time-course analysis of leaf SPAD index generally displayed lower values compared to plants grown at pH 6, independently from the Fe supply (Fig. 1A and 1C). Nevertheless, within the pH 8 growing condition, leaf SPAD index in +Fe plants was significantly higher with respect to that of -Fe plants (Fig. 1C). In these experimental conditions, the resupply with either Fe-biochelate or Fe-EDDHA did not produce a recovery in the chlorophyll content in treated plants, suggesting that at pH 8 these Fe sources might not be enough efficient (Fig. 1C); however, considering the demonstrated stability of Fe-EDDHA over a pH interval ranging from 3 to 10 (Lucena, 2006), the observed effects could be attributed to an extreme sensitivity of cucumber plants to the imposed pH in the nutrient solution impairing the functionality of the acquisition mechanism, as

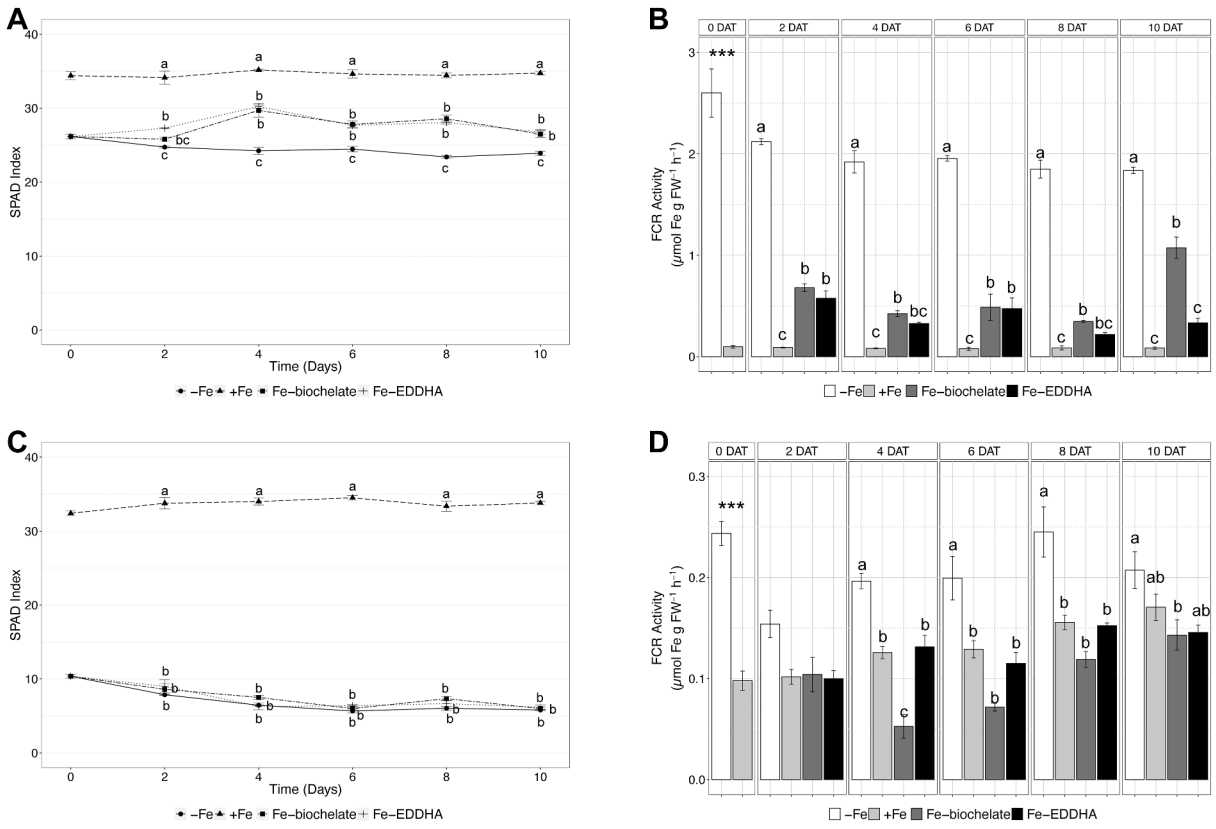

Fig. 1. Leaf SPAD index and root Fe-chelate reductase (FCR) activity of cucumber plants grown in nutrient solutions adjusted at pH 6 (A and B for leaf SPAD index and FCR, respectively) or pH 8 (C and D for leaf SPAD index and FCR, respectively) as affected by Fe treatments [Fe sufficient (+Fe), Fe deficient (-Fe), or Fe deficient and resupplied with Fe-biochelate or Fe-EDDHA] during the resupply experiment at different days after treatment (DAT). Measurements have been carried out on three independent biological replicates ($n = 3$) and reported as means ± standard errors. Within each sampling date, different letters indicate statistically different values ($P = 0.05$) by Tukey's test.

previously described (Cesco et al., 2002). This observation was further confirmed by the analysis of FCR activity (Fig. 1D); indeed, at 0 DAT, -Fe showed significantly higher FCR values as compared to +Fe plants, even though they were 10 times lower than those presented by -Fe plants grown at pH 6 (Fig. 1B and 1D). The resupply of -Fe plants with both Fe-biochelate and Fe-EDDHA caused a reduction in the FCR activity; interestingly, in the case of plants supplemented with Fe-EDDHA, the Fe reduction activity was statistically comparable to that observed in +Fe plants at all the time points considered. On the other hand, plants resupplied with Fe-biochelate showed FCR activity values even lower than +Fe plants, at least at 4 DAT (Fig. 1D). At 10 DAT, plants were harvested and both Fe concentration in plant tissues as well as the growth parameters were evaluated (Figs. 2 and 3). Concerning Fe concentration in root tissues, plants grown at pH 6 and resupplied with Fe-biochelate showed a significantly higher Fe concentration with respect to -Fe and Fe-EDDHA plants, albeit lower in comparison to +Fe plants (Fig. 2A). On the contrary, at pH 8 no significant difference could be highlighted among the different treatments, except for +Fe plants, which displayed the highest Fe concentration values (Fig. 2A). The Fe concentration in leaves, independently of the pH of the growth medium, did not display any significant variation, according with the treatments applied (Fig. 2B). The assessment of growth parameters showed that, at pH 6, +Fe plants had a 3-fold increase in the shoot dry weight (SDW) as compared to -Fe cucumbers; consistently with the results reported above, plants resupplied with either Fe-biochelate and Fe-EDDHA displayed a significant increase in the SDW (about 2-fold) with respect to Fe starved plants (Fig. 3A). The same trend has been also recorded for the root dry weight (RDW) (Fig. 3B), the total root length (Fig. 3C) and for the other parameters (i.e., root area, diameter and volume) describing the root system architecture (Supplementary Table1). When considering pH 8 growing condition, both +Fe and -Fe cucumber plants showed a reduction by about 50% in SDW and RDW as compared to plants grown at pH 6; nevertheless, the biomass of Fe sufficient plants was significantly higher with respect to -Fe cucumbers, at both root and shoot levels (Fig. 3A and B). In this case, differently from the previous one, the resupply with either Fe-biochelate or Fe-EDDHA did not produce any significant increase in the accumulation of biomass respect to -Fe plants (Fig. 3A and B). Same behavior has been also observed for the total root length (Fig. 3C) and for the other root architecture parameters (Supplementary Table1). To sum up, the whole dataset collected within the present experiment has been subjected to Principal Component Analysis, thereby obtaining a two components model explaining 78.77% of the total variance (Fig. 4). The main driver for sample clusterization was represented by the pH of the nutrient solution, as also previously discussed. However, among the different clusters, samples treated with Fe-biochelate and Fe-EDDHA grouped together, further confirming a similar fertilization potential for the two products investigated (Fig. 4).

2.2. *Case study 2*

The aim of Case Study 2 was to evaluate the efficacy of Fe-biochelate (KeyLan Fe) in comparison with Fe-EDDHA (Sequestrene Life) in enhancing yield and Fe nutrition of cherry tomato plants (*Solanum lycopersicum* L – cv Akira) grown in soilless culture under alkaline conditions (pH 8.0). Tomato plants (3-true leaf stage) were transplanted on 30 April into 9.5 L plastic pots filled with pure sand (particle size 2- 6 mm) arranged in single row at a plant density of 2.19 plants m^{-2}. Tomato plants were grown inside a 300 m^2 polyethylene greenhouse located at the Experimental Farm 'Nello Lupori' of Tuscia University, Viterbo. The greenhouse was maintained at daily temperature between

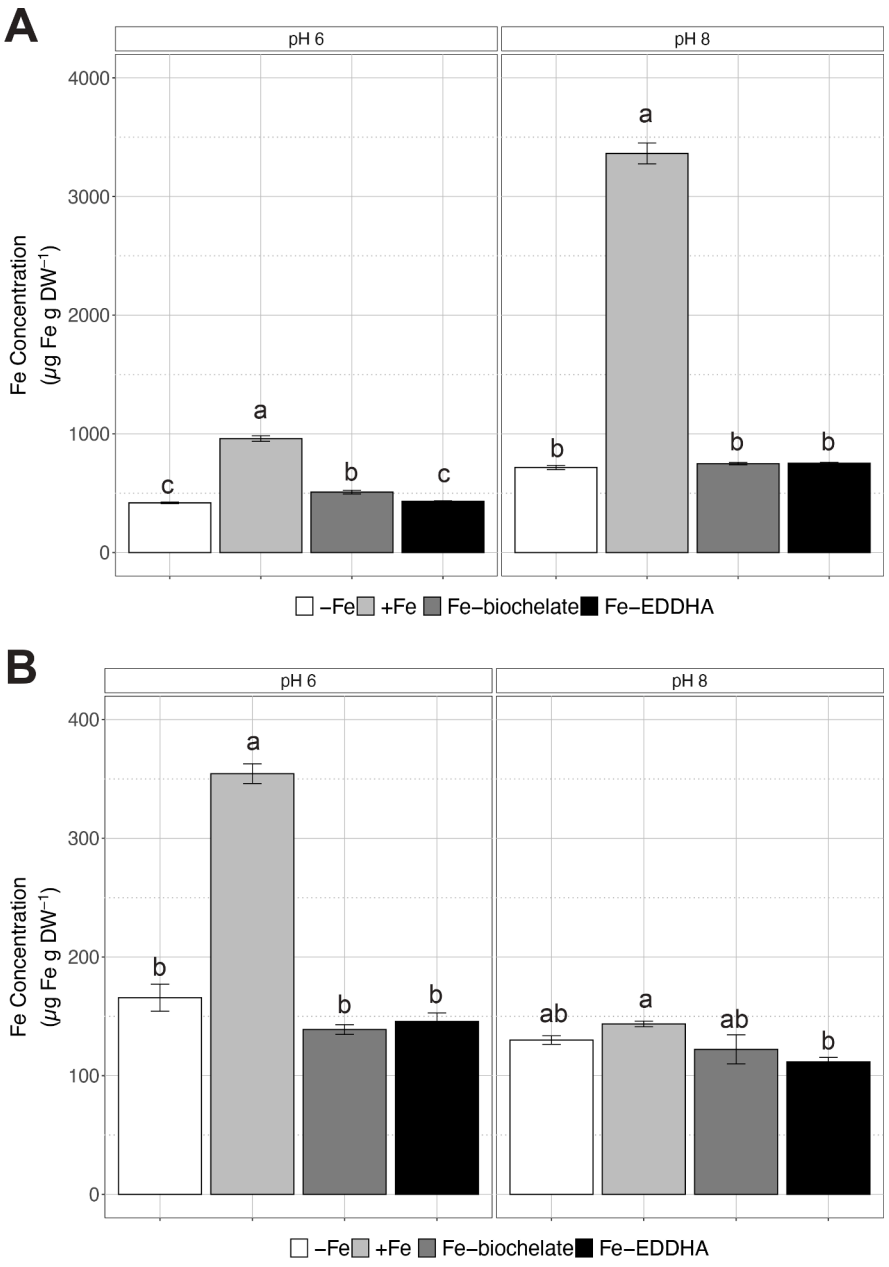

Fig. 2. Fe concentration in roots (A) and leaves (B) of cucumber plants grown in nutrient solutions adjusted at pH 6 or 8 as affected by Fe treatments [Fe sufficient (+Fe), Fe deficient (-Fe), or Fe deficient and resupplied with Fe-biochelate or Fe-EDDHA]. Measurements have been carried out on three independent biological replicates ($n = 3$) and reported as means ± standard errors. Different letters indicate statistically different values ($P = 0.05$) by Tukey's test.

18 and 33°C while night temperature was always above 16°C. Day/night relative humidity was between 55 and 85%. After transplant, tomato plants were drip-irrigated with a basic nutrient solution containing the following macronutrients (mM): 9 N—NO_3, 1.5 mM P, 1.5 mM S, 4.5 mM K, 2.5 Ca, and 2.0 mM Mg; while the micronutrients were provided as follow (μM): 3.3 Mn, 0.4 Cu, 0.6 Zn, 11.1 B, and 0.5 Mo. Moreover, the nutrient solution was titrated at pH 8.0 by adding 10 mM $NaHCO_3$ and 5 mM $CaCO_3$. In the three treatments, Fe was supplied every week through fertigation using the following rates: 16.0 mg Fe/plant as Fe-EDDHA (Full dose); 16.0 mg Fe/plant as Fe-biochelate (Full dose); 10.6 mg Fe/plant as Fe-biochelate (Reduced dose corresponding to 2/3 of the full dose). The experimental design was a randomized block design with 9 replicates per treatment. Tomato suckers were removed keeping one stem per plant and the main stem was pruned at the eighth truss. The trial ended on 19 August (111 DAT).

During the growing cycle SPAD index (SPAD-502, Minolta corporation, Ltd., Osaka, Japan) and chlorophyll fluorescence (Handy PEA - Hansatech Instruments Ltd, UK) were measured on the fourth-fully expanded leaves from the tops of the plants at 27, 40, 61 85 and 111 DAT. The maximum quantum yield of open photosystem II (PSII) (Fv/Fm) was calculated as reported by Maxwell and Johnson (2000).

Fully ripe fruits were harvested from 75 DAT until the termination of the experiment (111 DAT). The fruit yield, number of fruits and mean fruit weight of marketable and unmarketable fruits were recorded on all plants. At 75 and 89 DAT, nine full red ripe fruits were selected per plot to determine the fruit quality. Total soluble solids (°Brix) content and pH of the fruit juice was measured by an Atago N1 refractometer (Atago Co. Ltd., Japan) and pH-meter, respectively. Fruit dry matter was also determined after drying a sample of fruits in a forced-air oven at 65 °C until constant weight.

Dried leaf tissues were ground separately in a Wiley mill to pass through a 20-mesh screen, then 0.5 g of the dried leaf tissues were

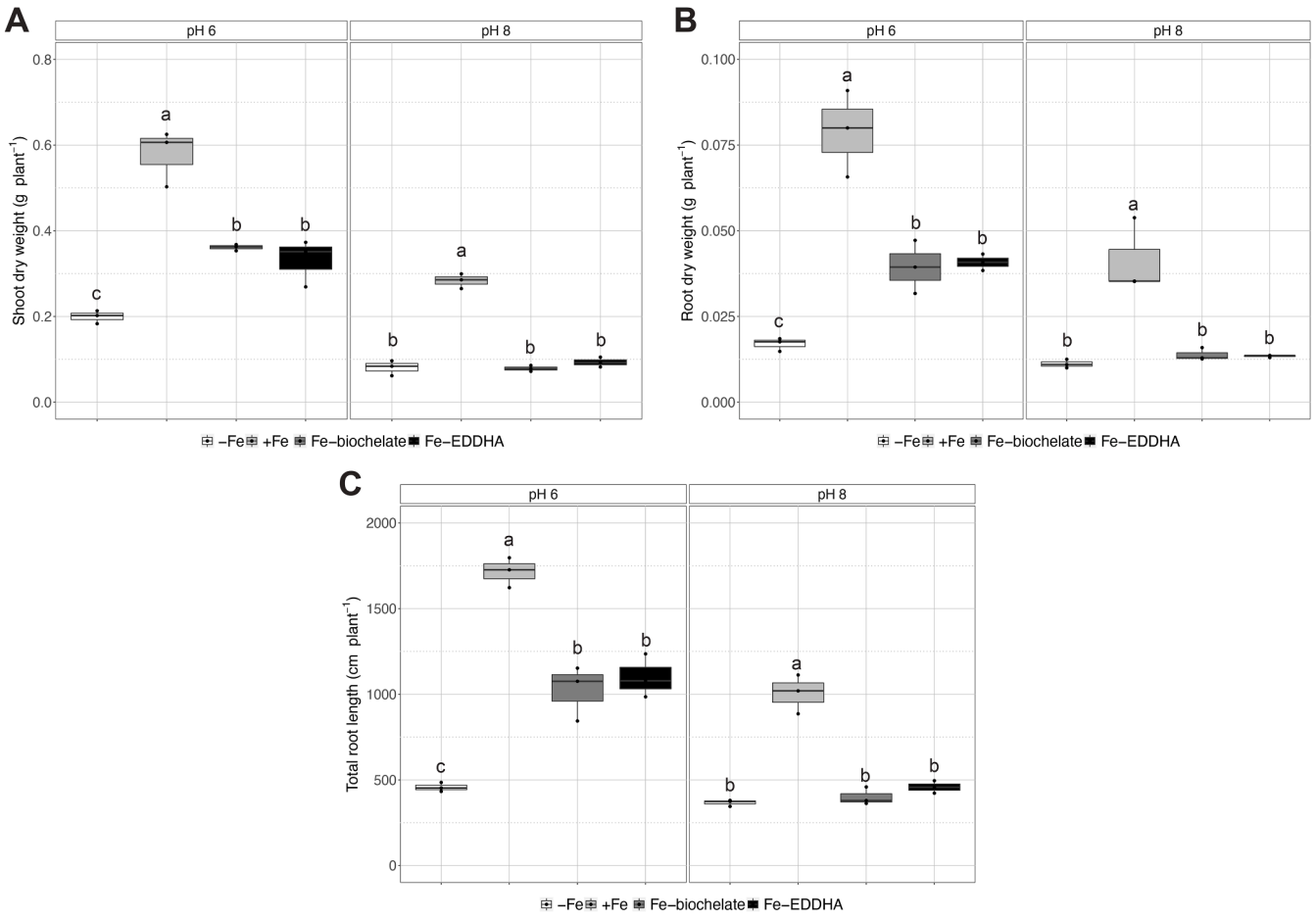

Fig. 3. Shoot dry weight (A), root dry weight (B), and total root length (C) of cucumber plants grown in nutrient solutions adjusted at pH 6 or 8 as affected by Fe treatments [Fe sufficient (+Fe), Fe deficient (-Fe), or Fe deficient and resupplied with Fe-biochelate or Fe-EDDHA]. Measurements have been carried out on three independent biological replicates ($n = 3$) and reported as means ± standard errors. Different letters indicate statistically different values ($P = 0.05$) by Tukey's test.

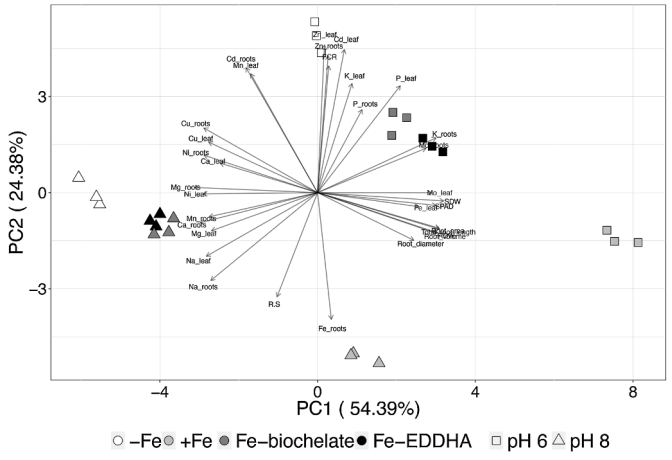

Fig. 4. Principal component loading plot and scores of principal component analysis of morphometric and biochemical traits and mineral contents in cucumber plants subjected at different Fe treatments [Fe sufficient (+Fe), Fe deficient (-Fe), or Fe deficient and resupplied with Fe-biochelate or Fe-EDDHA)] and nutrient solution pH (6 and 8).

analyzed for Fe concentration using an inductively coupled plasma emission spectrophotometer (ICP Iris; Thermo Optek, Milano, Italy). All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). To separate treatment means within each measured parameter, Tukey's multiple comparison test was performed at $P = 0.05$.

Leaf chlorophyll content was significantly affected only at the beginning of the crop cycle, where at 27 and 40 DAT, SPAD index was higher in leaves of plant treated with a full dose of Fe-EDDHA treatment and Fe-biochelate then with a reduced dose of Fe as Fe-biochelate (Table 1).

Similarly to SPAD index results, the maximum quantum yield of PSII in the leaves was significantly affected by Fe treatments only at the beginning of the crop cycle with highest values with Fe-EDDHA. However, these differences among treatments disappeared after the first measurements until the end of the trial. Leaf Fe concentration at the end of the trial was not significantly affected by treatments (avg. 135.3 mg

Table 1
Effects of Fe treatments on SPAD index of tomato leaves at different days after transplanting (DAT).

Treatment	SPAD Index				
	27 DAT	40 DAT	61 DAT	85 DAT	111 DAT
Fe-EDDHA (Full dose)	58.1 a	65.1 a	61.4	64.9	68.3
Fe-biochelate (Full dose)	56.8 ab	67.0 a	59.1	62.3	68.2
Fe-biochelate (Reduced dose)	55.8 b	62.3 b	60.0	66.2	70.4
Significance	*	***	ns	ns	ns

ns, *, ***Nonsignificant or significant at $P<0.05$, or 0.001, respectively. Means within columns separated using Tukey's multiple comparison test, $P = 0.05$.

Fe/kg d.wt.). Moreover, crop yield and yield components were not significantly affected by Fe treatments (avg. 983.2 g of marketable yield per plant; 89.7 marketable fruits per plant; 11.0 g per marketable fruit; 1308.2 g of total yield per plant; 135.1 total fruits per plant). Quality traits of marketable fruits such as total soluble content, pH and fruit dry matter measured at 75 and 89 days after transplanting were also not significantly affected by treatments (avg. 7.7 and 7.9 °Brix; 4.4 and 4.4 pH; 10.8 and 9.4% of dry matter, respectively). The above findings demonstrated that both Fe sources can be used to improve Fe nutrition and agronomic performances of tomato crop under alkaline conditions. Moreover, the similar agronomic performances of tomato plants fertilized with full and reduced dose of Fe suggest the possibility to reduce the Fe supply to the plants using Fe-biochelate.

2.3. Case study 3

The aim of Case Study 3 was to evaluate the efficacy of Fe-biochelate (KeyLan Fe) and Fe-EDDHA (Sequestrene Life) at two Fe rates (1.46, and 2.92 mg plant^{-1}) in enhancing yield and Fe nutrition of strawberry plants (*Fragaria x ananassa* – cv Monterey) grown in soilless culture under optimal and alkaline conditions (pH 6.5 and 8.0, respectively). Strawberry plants (3-true leaf stage) were transplanted on 16 February into 1.5 L plastic pots filled with a mixture of pure sand (particle size 2-6 mm) and field soil (0.7:0.3 v/v) arranged in single row at a plant density of 5.0 plants m^{-2}. Strawberry plants were grown inside a 300 m^2 polyethylene greenhouse located at the Experimental Farm 'Nello Lupori' of Tuscia University, Viterbo. The greenhouse was maintained at daily temperature between 16 and 32°C while night temperature was always above 12°C. Day/night relative humidity was between 55 and 85%. After transplant, plants were drip-irrigated with a basic nutrient solution containing the following macronutrients (mM): 9.0 N—NO_3, 1.5 mM P, 1.5 mM S, 4.5 mM K, 2.5 Ca, and 2.0 mM Mg; while the micronutrients were provided as follow (μM): 3.3 Mn, 0.4 Cu, 0.6 Zn, 11.1 B, and 0.5 Mo. The alkaline solution (pH 8.0) was obtained by adding 10 mM $NaHCO_3$ and 5 mM $CaCO_3$ to the basic nutrient solution. Fe fertilization was made every week starting from 4 weeks after transplanting. For both Fe sources, Fe fertilizer rate was supplying in water solution by dissolving the Fe rate per plant in 50 ml of water. The experimental design was a factorial design (2 Fe sources x 2 Fe rates x 2 pH levels) in a randomized block design with 4 replicates per treatment. Each plot was composed by five plants. Plants were drip-irrigated as needed with emitters having a flow rate of 2 L h^{-1}. The fruit harvest was performed from 22 April to the end of the trial (9 May - 83 DAT). During the growing cycle, chlorophyll index (Multi-Pigment-Meter MPM-100 - ADC BioScientific Ltd., UK) and chlorophyll fluorescence (Handy PEA - Hansatech Instruments Ltd, UK) were measured on the top fully expanded leaves at 40, 48, 55, 63, 70, and 76 DAT. The maximum quantum yield of open photosystem II (PSII) (Fv/Fm) was calculated as reported by Maxwell and Johnson (2000).

Fully ripe fruits were harvested from 60 DAT to the end of the experiment. The fruit yield, number of fruits and mean fruit weight of marketable (fruit diameter>25 mm) and unmarketable fruits (fruits having a diameter<25 mm, rotten fruits and deformed fruits) were recorded in all plants. At 65, and 72 DAT, four full red ripe fruits were selected per plot to determine the fruit quality. Total soluble solids content of the fruit juice was measured by an Atago N1 refractometer (Atago Co. Ltd., Japan) and expressed as °Brix. Fruit dry matter was also determined after drying a sample of fruits in a forced-air oven at 65 °C until constant weight.

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). To separate treatment means within each measured parameter, Tukey's multiple comparison test was performed at $P = 0.05$.

During the growing cycle, leaf chlorophyll index was significantly affected mainly by Fe source and pH (Table 3) with highest values in pH 6.0 treatment at 40, 48, 55, 63 and 76 DAT, and in Fe-EDDHA treatment at 40, 48, 55, 63, 70 and 76 DAT. Moreover, maximum quantum yield of PSII (Fv/Fm) of strawberry leaves was significantly affected only by Fe source and pH value at the end of the trial (Table 3); Fv/Fm values were highest in Fe-biochelate treated plants and in pH 6.0 treatment at both 70 and 76 DAT (Table 3). Total, marketable and unmarketable yield of strawberry fruits were not significantly affected by treatments (data not shown): the average values were 493, 474, and 19 g plant^{-1}, respectively. Fruit quality (dry matter and total soluble solids) was not significantly affected by treatments at both sampling dates (data not shown): the average values of dry matter and total soluble solids were 9.4 and 11.5%, and 5.7 and 6.5°Brix, respectively. The above findings showed that the enhancement of leaf chlorophyll index in Fe-EDDHA treated plants at both pH values (6.0 and 8.0) has not resulted in an increase of the quali-quantitative traits of strawberry fruits, being similar in both Fe-source treatments. Interestingly, root applications of Fe-biochelate enhanced the maximum quantum yield of PSII (Fv/Fm) in the strawberry leaves at the end of the trial indicating a better potential photosynthetic capacity in comparison with Fe-EDDHA. The above findings highlight that the environmentally-friendly Fe-biochelate could be a good substitute of Fe-EDDHA for Fe nutrition of strawberry plants (Table 2).

3. Conclusions

Nowadays, sustainable agriculture has become an imperative to meet the needs of present and future generations, while ensuring profitability, environmental health, and social and economic equity. To be sustainable it is necessary to use innovative and environmentally friendly technical solutions like plant biostimulants. Plant biostimulants have been combined with mineral nutrients to enhance plant nutrient uptake and assimilation processes. This approach has been used in the development of metal-biochelates which combine mineral cation nutrients and bioactive peptides. Starting from the above considerations, a Fe-biochelate has been compared with Fe-EDDHA in three trials on cucumber, tomato and strawberry under limiting Fe availability. Results of trials demonstrated that Fe-biochelate was as effective as Fe-EDDHA to meet the Fe needs of tested crops even under circumneutral (pH 6.0) and extreme alkaline conditions (pH 8.0). Considering the potential negative impact of synthetic chelates on the environment and the long persistence of these compounds in the plant tissues, the above research findings are of great interest to enhance the sustainability of crop production. Finally, Fe-biochelate could be used in soilless culture as an alternative to synthetic Fe chelates for Fe enrichment of edible plant tissues (biofortification) increasing their nutritional value. Such approach may also be used for other minerals (e.g., Zn, Mn) having a positive impact on human nutrition and health.

CRediT authorship contribution statement

Monica Yorlady Alzate Zuluaga: Writing – original draft, Visualization, Investigation, Data curation, Methodology, Validation, Writing –

Table 2
Effects of Fe treatments on maximum quantum yield of PSII (Fv/Fm) of tomato leaves at different days after transplanting (DAT).

Treatment	Fv/Fm				
	27 DAT	40 DAT	61 DAT	85 DAT	111 DAT
Fe-EDDHA (Full dose)	0.745 a	0.822	0.780	0.763	0.804
Fe-biochelate (Full dose)	0.698 b	0.816	0.792	0.775	0.802
Fe-biochelate (Reduced dose)	0.714 b	0.806	0.785	0.742	0.819
Significance	**	ns	ns	ns	ns

ns, **Nonsignificant or significant at $P<0.01$, respectively. Means within columns separated using Tukey's multiple comparison test, $P=0.05$.

Table 3
Effects of Fe source, Fe rate and pH treatments on chlorophyll index and maximum quantum yield of PSII (Fv/Fm) of strawberry leaves at different days after transplanting (DAT).

Fe source	Fe rate (mg/plant)	pH	Chlorophyll index						Fv/Fm					
			40 DAT	48 DAT	55 DAT	63 DAT	70 DAT	76 DAT	40 DAT	48 DAT	55 DAT	63 DAT	70 DAT	76 DAT
Fe-EDDHA	1.46	6.0	0.87	0.73	0.85	0.75	0.73	0.75	0.80	0.80	0.75	0.76	0.76	0.78
		8.0	0.75	0.82	1.08	0.62	0.73	0.69	0.81	0.77	0.77	0.74	0.71	0.72
	2.92	6.0	0.83	0.65	0.90	0.76	0.75	0.74	0.80	0.75	0.73	0.73	0.77	0.75
		8.0	0.81	0.82	0.99	0.65	0.70	0.64	0.81	0.77	0.72	0.63	0.72	0.69
Fe-biochelate	1.46	6.0	0.79	0.69	0.92	0.68	0.66	0.67	0.79	0.78	0.77	0.75	0.78	0.82
		8.0	0.70	0.63	0.95	0.52	0.44	0.41	0.80	0.75	0.71	0.72	0.74	0.73
	2.92	6.0	0.75	0.60	0.77	0.67	0.64	0.63	0.81	0.78	0.71	0.77	0.79	0.80
		8.0	0.70	0.71	0.92	0.48	0.44	0.40	0.81	0.76	0.70	0.72	0.75	0.74
Significance														
Fe source (A)			**	***	*	***	***	***	NS	NS	NS	NS	*	**
Fe rate (B)			NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
pH			***	**	***	***	NS	***	NS	NS	NS	NS	*	*
$A \times B$			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$A \times$ pH			NS	*	NS	NS	NS	***	NS	NS	NS	NS	NS	NS
$B \times$ pH			NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
$A \times B \times$ pH			NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS

ns, *, **, ***Nonsignificant or significant at $P<0.05$, 0.01 and 0.001, respectively.

review & editing. **Mariateresa Cardarelli:** Writing – original draft, Visualization, Investigation, Data curation, Methodology, Validation, Writing – review & editing. **Youssef Rouphael:** Investigation, Writing – review & editing. **Stefano Cesco:** Supervision, Writing – review & editing. **Youry Pii:** Conceptualization, Visualization, Investigation, Supervision, Validation, Writing – review & editing. **Giuseppe Colla:** Conceptualization, Visualization, Investigation, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.111833.

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