Brief characterisation of Fe chlorosis in chia (*Salvia hispanica* L.) plants grown in nutrient solution

João Vieira, Luna Džidić-Uzelac, Izabela Thais dos Santos, Marco Patrício, Florinda Gama, Teresa Saavedra, Pedro José Correia & Maribela Pestana

Published online: 26 Nov 2023.

Submit your article to this journal

Article views: 22

View related articles

View Crossmark data

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Check for updates

Brief characterisation of Fe chlorosis in chia (*Salvia hispanica* L.) plants grown in nutrient solution

João Vieira[a,b], Luna Džidić-Uzelac[b], Izabela Thais dos Santos[b,c], Marco Patrício[b], Florinda Gama[b,d], Teresa Saavedra[a,b], Pedro José Correia[a,b] and Maribela Pestana ![ORCID][a,b]

[a]MED — Mediterranean Institute for Agriculture, Environment and Development and CHANGE—Global Change and Sustainability Institute, University of Algarve, Faro, Portugal; [b]Faculty of Science and Technology, University of Algarve, Faro, Portugal; [c]UNESP — São Paulo State University, Botucatu, Brasil; [d]GreenCoLab — Associação Oceano Verde, University of Algarve, Faro, Portugal

ABSTRACT

Chia (*Salvia hispanica* L.) plant is a well-known plant due to the nutraceutical value of its seeds. The aim of this preliminary study was to assess the response of Chia plants to Fe deficiency. Chia plants were grown for 12 days in Hoagland's nutrient solutions without Fe (Fe0) and with Fe (Fe10-10 µM Fe). Biomass parameters and root ferric chelate-reductase activity (FC-R; EC 1.16.1.17) were determined at the end of the experiment. Chlorophyll estimations (expressed as SPAD readings) decreased progressively, showing the typical symptoms of iron chlorosis. In addition, iron-deficient chia plants exhibit smaller biomass (number of leaves, root, and shoot growth reduction) compared to control plants. These plants also showed morphological changes in roots. Furthermore, root FC-R activity was significantly lower in Fe0 plants.

Introduction

Nowadays, due to the change people's lifestyle and their willingness to learn more about the benefits of bioactive ingredients in the diet, functional foods are gaining more popularity, especially in highly developed countries.

Chia (*Salvia hispanica* L.) is an annual herbaceous plant from the Lamiaceae family, originated in mountainous areas of Mexico and Guatemala (Lovelli et al. 2019). The seeds of chia have gained popularity and are often referred to as a 'superfood' due to their exceptional nutritional profile and potential health benefits, particularly their high content of essential unsaturated fatty acids and dietary fibre, as well as essential amino acids, polyphenols, vitamins, and nutrients (Martínez-Cruz and Paredes-López 2014; Motyka et al. 2023; Orona-Tamayo et al. 2017). These characteristics have encouraged increased crop production worldwide. Currently, the largest production centre for chia seeds is in Bolivia, Argentina, Ecuador, and Guatemala (Ayerza 2009) and possibilities for the production of chia in other parts of the world are still being investigated (Amato et al. 2015).

There are several studies about the chemical and nutritional composition of chia seeds, but the information about vegetative and ecophysiological traits of this is scarce. The

---

CONTACT Maribela Pestana ✉ fpestana@ualg.pt

increase in demand for chia seeds will induce the expansion of chia cultivation to eda-phoclimatic environments sensitive to climate change, which may affect nutritional avail-ability in several ways. Calcareous soils predominate in areas that may be subject to extreme weather episodes and nutritional imbalances, and among these, iron (Fe) chloro-sis in plants is the most prevalent in those soils and remains unresolved.

Fe is an essential micronutrient for all higher organisms, and its deficiency causes a serious nutritional problem in both humans and plants (Ofori et al. 2022; Zuluaga et al. 2023). In plants, Fe has an important role in various metabolic pathways including photosynthesis, respiration, chlorophyll biosynthesis, and several redox reactions (Vélez-Bermúdez and Schmidt 2022; 2023).

Iron deficiency results in a decrease in the concentration of photosynthetic pigments in leaves, usually referred to as Fe chlorosis (Abadía and Abadía 1993). The symptoms of Fe deficiency occur primarily in young leaves and became apparent as an interveinal chlorosis with the appearance of a fine reticulation (Pestana et al. 2004).

Under deficiency conditions, plants have two Fe acquisition strategies referred as Strat-egy I and Strategy II (Marschner et al. 1986), which are not mutually exclusive (Nozoye et al. 2017; Rajniak et al. 2018). Strategy I-plants found in dicotyledonous plants and non-grasses have several mechanisms to increase Fe uptake, which include proton extru-sion to rhizosphere, and increased activity of root ferric chelate-reductase activity, an enzyme that reduces Fe(III) to Fe(II) (Römheld 1987). Strategy II-plants rely on the secretion of phytosiderophores into the rhizosphere together with the induction of a high-affinity system for Fe(III)–phytosiderophore uptake (Marschner et al. 1986).

Phylogenetically, chia plants belong to the Strategy I group, however, the limit between the strategies is currently questioned, making it necessary to characterise the mechanisms of response of plant species recently introduced in agricultural production (Vélez-Bermúdez and Schmidt 2022).

In this context, this crop deserves further attention regarding the response to nutri-tional deficiencies, so the main aim of this study was to analyse, for the first time, the influence of Fe deficiency on the vegetative and physiological performance of chia plants grown in nutrient solution.

Materials and methods

The experiment was conducted in a glasshouse at the University of Algarve, Portugal (latitude: 37°04'35''N, longitude: 7°97'51'' W) under natural photoperiod conditions: a photosynthetic photon flux density (PPFD) of $150-450$ mol m^{-2} s^{-1} at the top of the plants, the air temperature was $\leq 25°C$ and the average relative humidity of 65%. Seeds of chia (*Salvia hispanica* L.) were germinated in a mixture of vermiculite and peat (1:2 v/v) and grown in a greenhouse and twenty-six days after, young plants were transferred to glass containers with 1L of Hoagland's nutrient solution (in mM): 2.5 $Ca(NO_3)_2.4H_2O$, 2.5 KNO_3, 0.5 KH_2PO_4, 1.0 $MgSO_4.7H2O$, and (μM): 23.0 H_3BO_3, 4.5 $MnCl_2.4H_2O$, 0.4 $ZnSO_4.7H_2O$, 0.2 $CuSO_4.5H_2O$, 1.0 MoO_3. Iron was supplied as Fe(III)-EDDHA (Basafer® from Compo, with 6% of Fe; 5.0% of Fe chelated by ortho–ortho EDDHA) at two concentrations: 0.0 (Fe0; without Fe) or 10.0 μM of Fe (Control; complete nutrient solution). At least five plants were used for each treatment in a completely randomised design. Glass containers were wrapped in non-transparent

black plastic bags to protect the roots from the light. Roots were continuously aerated using an air pump and aeration tubes. At the beginning of the experiment, the pH of nutrient solutions was adjusted to 6.0 ± 0.1 using a NaOH 0.1 M aqueous solution and their electrical conductivity (EC) was 2.2 ± 0.1 dS m^{-1}. The pH and EC of solutions were monitored daily, and solutions were renewed when the values were less than 2.0 ± 0.1 dS m^{-1}. The experiment was performed during 12 days of November under natural photoperiod conditions. The air temperature was $\leq 20°C$ and the average relative humidity was 65%.

The number of leaves per plant was recorded during the experiment. The portable SPAD-502 apparatus (Minolta Corp., Osaka, Japan) was used to, non-destructively, evaluate the leaf chlorophyll concentration in young and mature leaves during the experiment. Data of mature leaves was not shown since no differences were observed (25.8 ± 1.0). At the end of the experiment, the plant material was separated into shoots and roots, and the fresh (FW) and dry weight (DW) of each part was determined after drying at 60° C until constant weight.

At the end of the experiment, ferric chelate–reductase (FC-R; EC 1.16.1.17) activity was measured in a single root tip, with approximately 2 cm of length (1.7 ± 0.7 mg of fresh weight – FW), by the formation of the Fe(II)-BPDS (bathophenantroline disulfonate) complex from Fe(III)-EDTA, as in Bienfait et al. (1983). Root tips of the distal part of the roots were excised with a razor blade from at least three plants of each treatment. Each root tip was incubated in an Eppendorf tube in the darkness and for one hour, with 900 µL of micronutrient-free half-strength Hoagland's nutrient solution, containing 300 µM BPDS, 500 µM Fe(III)-EDTA and 5 mM MES, pH 6.0. Then, the FC-R activity was measured at 535 nm, using a spectrophotometer (CADAS 100 UV-VIS Photometer; Dr. Lange, Düsseldorf, Germany), using a molar extinction coefficient of 22.14 mM^{-1} cm^{-1}. Blank controls without root tips were also used to correct for any non-specific photoreduction. The total number of apices was recorded in each root tip (2 cm length). The FC-R activity was expressed on a FW basis per root tip and per apex of each root tip (Pestana et al. 2011).

Plant data were analysed through one-way analysis of variance (ANOVA; F test). Differences were considered significant at $P < 0.05$. (IBM SPSS® software, version 20).

Results

In the initial development, all chia plants (*Salvia hispanica* L.) were green and healthy, with mean SPAD values of 29.7 ± 0.5, and a mean height of 19.0 ± 1.3 cm, with 8 leaves per plant, and a shoot and root FW of 2.2 ± 0.6 g and 2.0 ± 0.5 g, respectively.

Control chia plants remained green throughout the experimental period with SPAD values varying between 29.7 and 31.8 in young leaves. After five days, plants grown without Fe showed typical slight symptoms of Fe deficiency chlorosis (Figure 1). Afterwards (9 days), young leaves were characterised by general yellowing of the mesophyll. At the end of the experiment (day 12), necrosis was also observed in plants of Fe0 treatment. The roots of the control plants were thin and dense, while the roots of the Fe0 plants were sparse, with a subapical root swelling and lateral roots (Figure 1).

As expected, SPAD values of young leaves were always significantly higher in Control plants than in Fe0 plants. After 12 days of growth without Fe, Fe0 plants reached
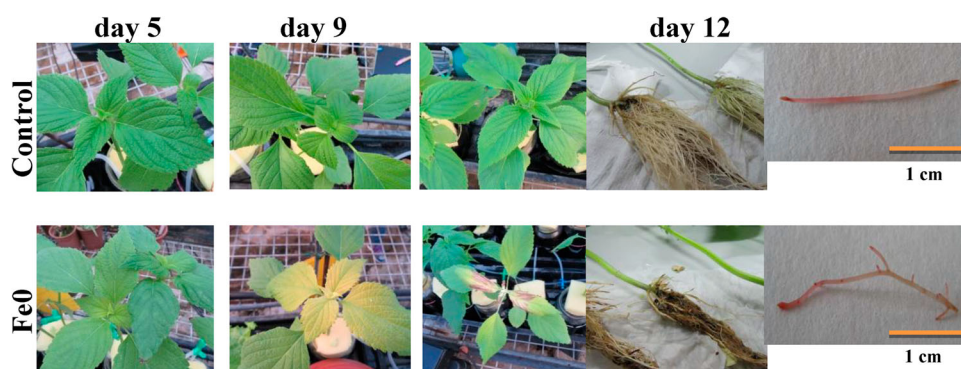
Figure 1. Effect of Fe depletion on Chia plants (in days 5, 9, and 12 of the experiment) compared to control plants. At day 12 roots appearance and root apexes are also shown; The rose colouration of root tips indicates FC-R activities. Fe treatments: Fe0 – without Fe in nutrient solution; Control – with Fe in the nutrient solution.

minimum values of SPAD, which correspond to a decrease of 79.3%, compared to control plants (Figure 2A). Until day 9, the number of leaves from Fe0 treatment was not significantly different from those of the controls (Figure 2B). The decrease in the number of leaves was less pronounced, showing a decrease of 25.3%, only in the last review.

At the end of the experiment, the total biomass (FW) was higher, about 1.8 times, in the control plants (9.7 ± 1.8 g) compared to the Fe0 plants (5.4 ± 1.5 g). The same tendency is maintained when expressing the biomass in DW (Figure 2C). The root-to-shoot ratio (FW and DW) was not significantly affected by Fe deficiency, considering the total time of growing days.

At the end of the experiment, all plants growing in the absence of Fe showed subapical root swelling, and this feature was absent in the Control plants. The number of root apexes was significantly higher (5 times) in plants grown without Fe, however, a lower root FC-R (nmol Fe(II) $\min^{-1}$ g^{-1} FW per root apex) activity per root apex was recorded by 4.5 times compared to Control plants (Figure 2D).

Discussion

Total chlorophyll content of young leaves, expressed by SPAD readings, was significantly affected by Fe deficiency resulting in the appearance of Fe chlorosis (young leaves with interveinal chlorosis and necrotic zones on the edges of the blade and old green leaves), as observed in several other species (de la Guardia and Alcántara 2002; Saavedra et al. 2022; Jelali et al. 2010; Pestana et al. 2005). It is well known that Fe deficiency affects the development and function of chlorophyll (Abadía and Abadía 1993), but this is the first time, as far as we know, that this result has been reported in chia plants.

Under Fe deficiency conditions (Fe0), plants had a smaller number of leaves, less root and shoot growth however, the root-to-shoot ratio was not affected in our experimental conditions. This ratio is commonly used to assess the distribution of photoassimilates, and iron-deficient plants often exhibit increased root-to-shoot ratios than in the controls due to the decreased shoot dry weight, and not to increased root biomass as described for
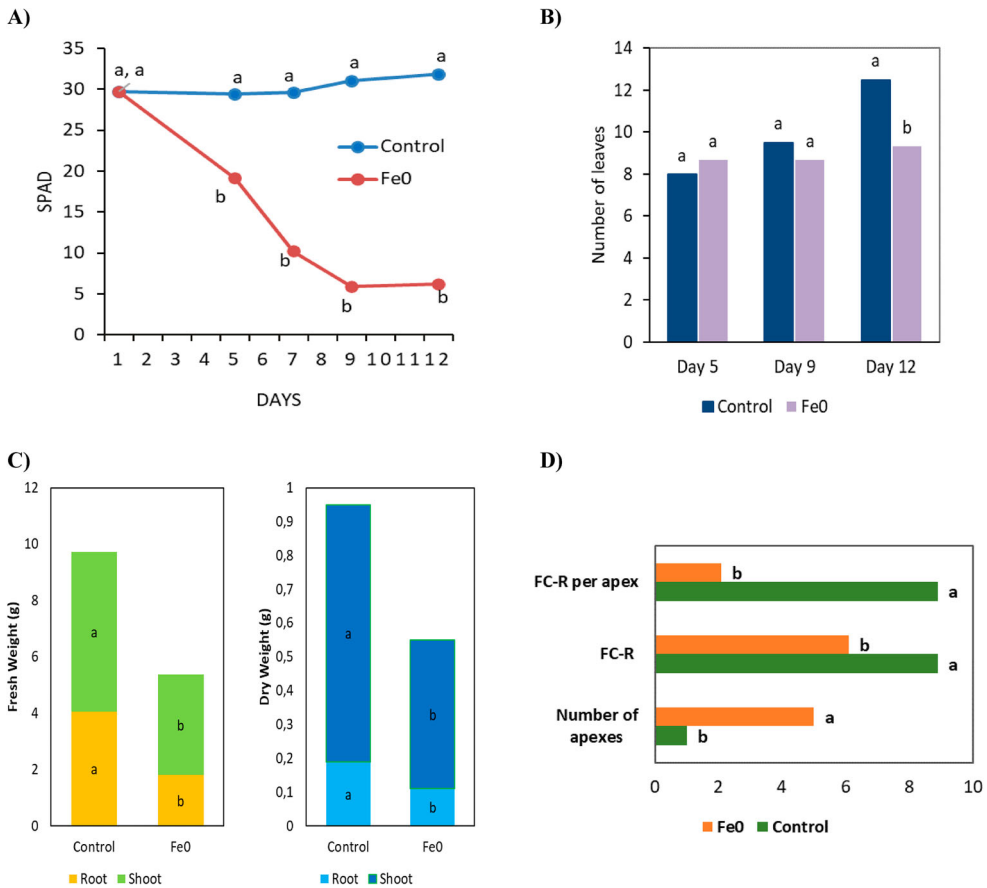
Figure 2. A, SPAD values of young leaves and **B,** number of leaves per plant during the experimental period. **C,** Biomass parameters at the end of experiment (day 12). **D,** Number of root apexes and root ferric chelate–reductase (FC-R) activity expressed in nmol Fe(II) min^{-1} g^{-1} FW at the end of the experiment (day 12). Treatments were: Fe0 – without Fe in nutrient solution and Control – with Fe in the nutrient solution. For each parameter analysed, different letters represent significant differences between samples ($P < 0.05$).

other species (McDonald et al. 1996; Pestana et al. 2011). Apparently, chia plants were able to balance resources allocation between shoots and roots under Fe depletion, despite the similar value of root DW. However, since some slight chlorosis symptoms were observed within five days, it seems that this species is highly sensitive to Fe deficiency. Chia seedlings are also very sensitive to salt stress and NaCl concentrations above 10 mM induced significant physiological damage (Younis et al. 2021).

In the present work, the roots of plants grown with Fe were thin and dense with a normal morphology. However, roots of chlorotic plants showed morphological changes typical of Fe deficiency in dicotyledonous plants (Strategy-I such as lateral root ramifications already described in strawberry, cucumber, sugar beet, orange, and carob tree (Correia et al. 2003; Jiménez et al. 2019; Landsberg 1982; Pestana et al. 2001; Romera and Alcantara 1994). The function of mentioned changes is to increase the surface area between the root and the soil as well as the availability of iron for

uptake. This morphological change may amplify the reduction points in roots allowing a higher Fe uptake (Gama et al. 2016; Pestana et al. 2011; Thimm et al. 2001).

When plants experience iron deficiency, they upregulate FC-R activity, normally leading to a higher rate of ferric iron reduction at the root surface (e.g. Gama et al. 2023; Jelali et al. 2010; Pestana et al. 2004, 2005). In contrast with this evidence, roots of Fe-deficient Chia plants showed lower FC-R activity, either expressed per g of root FW or per number of root apexes. This result is probably the most important outcome of our work. In the literature, results referring to low FC-R activity in Fe-deficient plants are scarce. In the same way, soybean (*Glycine max*) is strongly affected by Fe depletion (Merry et al. 2022; Vasconcelos et al. 2014). It is likely that Chia is sensitive to Fe depletion, but it would be necessary to evaluate the effect of small amounts of Fe on the activity of the enzyme. Besides, other previous work by Gogorcena et al. (2000) showed that in vivo FC-R activity in a peach rootstock changed over time, but it is not possible to prove this hypothesis as we did not measure the time-course of the enzyme. Although the results point to Chia being an inefficient species, as it does not show an increase in FCR activity in the absence of Fe, further research is necessary.

In conclusion, the results presented in the study indicate that iron deficiency negatively affected shoot vegetative performance and total chlorophyll content. Despite the morphological root changes, root biomass (expressed as DW) did not decrease significantly in chlorotic plants suggesting an optimisation of resources allocation. Chia is a relatively new commercial food crop that deserves further attention, particularly regarding the effects of abiotic stress on seed quality.

Author contributions

MP and PJC conceived the idea and designe the experiment. LD, IS, MP, FG and TS performed the experiment and collected the data. All authors analysed and discussion the data. JV, LD, MP and PJC wrote the paper. All authors read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Maribela Pestana 🄳 http://orcid.org/0000-0002-4319-1682

References

Abadía J, Abadía A. 1993. Iron and pigments. In: Barton LL, Hemming BC, editors. Iron chelation in plants and soil microorganisms. San Diego, CA, USA: Academic Press; p. 327–343.

Amato M, Caruso MC, Guzzo F, Galgano F, Commisso M, Bochicchio R, Labella R, Favati F. 2015. Nutritional quality of seeds and leaf metabolites of Chia (*Salvia hispanica* L.) from southern Italy. European Food Research and Technology. 241:615–625. doi:10.1007/s00217-015-2488-9.

Ayerza R. 2009. The seed's protein and oil content, fatty acid composition, and growing cycle length of a single genotype of chia (*Salvia hispanica* L.) as affected by environmental factors. Journal of Oleo Science. 58:347–354. doi:10.5650/jos.58.347.

Bienfait HF, Bino RJ, Van der Blick AM, Duivenvoorden JF, Fontaine JM. 1983. Characterization of ferric reducing activity in roots of Fe-deficient *Phaseolus vulgaris*. Physiologia Plantarum. 59:196–202. doi:10.1111/j.1399-3054.1983.tb00757.x.

Correia PJ, Pestana M, Martins-Loução MA. 2003. Nutrient deficiencies in carob (*Ceratonia siliqua* L.) grown in solution culture. The Journal of Horticultural Science and Biotechnology. 78:847–852. doi:10.1080/14620316.2003.11511708.

de la Guardia MD, Alcántara E. 2002. A comparison of ferric-chelate reductase and chlorophyll and growth ratios as indices of selection of quince, pear and olive genotypes under iron deficiency stress. Plant and Soil. 241:49–56. doi:10.1023/A:1016083512158.

Gama F, Saavedra T, Dandlen S, García-Caparrós P, de Varennes A, Nolasco G, Correia PJ, Pestana M. 2023. Silencing of *FRO1* gene affects iron homeostasis and nutrient balance in tomato plants. Journal of Plant Nutrition and Soil Science. 186:554–567. doi:10.1002/jpln.202300071.

Gama F, Saavedra T, Gonzalez A, Miguel MG, de Varennes A, Miguel P, Pestana M. 2016. The memory of iron stress in strawberry plants. Plant Physiology and Biochemistry. 104:36–44. doi:10.1016/j.plaphy.2016.03.019.

Gogorcena Y, Abadía J, Abadía A. 2000. Induction of *in vivo* root ferric chelate reductase activity in fruit tree rootstock. Journal of Plant Nutrition. 23:9–21. doi:10.1080/01904160009381993.

Jelali N, Dell'Orto M, Rabhi M, Zocchi G, Abdelly C, Gharsalli M. 2010. Physiological and bio-chemical responses for two cultivars of Pisum sativum ("Merveille de Kelvedon" and "Lincoln") to iron deficiency conditions. Scientia Horticulturae. 124:116–121. doi:10.1016/j.scienta.2009.12.010.

Jiménez MR, Casanova L, Saavedra T, Gama F, Suárez MP, Correia PJ, Pestana M. 2019. Responses of tomato (*Solanum lycopersicum* L.) plants to iron deficiency in the root zone. Folia Horticulturae. 31(1):223–234. doi:10.2478/fhort-2019-0017.

Landsberg E-. 1982. Transfer cell formation in the root epidermis: A prerequisite for fe-efficiency? Journal of Plant Nutrition. 5:415–432. doi:10.1080/01904168209362970.

Lovelli S, Valerio M, Phillips TD, Amato M. 2019. Water use efficiency, photosynthesis, and plant growth of Chia (*Salvia hispanica* L.): a glasshouse experiment. Acta Physiologiae Plantarum. 41. doi:10.1007/s11738-018-2795-4.

Marschner H, Römheld V, Kissel M. 1986. Different strategies in higher plants in mobilization and uptake of iron. Journal of Plant Nutrition. 9:693–713. doi:10.1080/01904168609363475.

Martínez-Cruz O, Paredes-López O. 2014. Phytochemical profile and nutraceutical potential of chia seeds (*Salvia hispanica* L.) by ultra high performance liquid chromatography. Journal of Chromatography A. 1346:43–48. doi:10.1016/j.chroma.2014.04.007.

McDonald AJS, Ericsson T, Larsson C-M. 1996. Plant nutrition, dry matter gain and partitioning at the whole-plant level. Journal of Experimental Botany. 47:1245–1253. doi:10.1093/jxb/47.Special_Issue.1245.

Merry R, Dobbels A-A, Sadol W, Naeve S, Stupar R-M, Lorenz A-J. 2022. Iron deficiency in soybean. Crop Science. *62*:36–52. doi:10.1002/csc2.20661.

Motyka S, Skała E, Ekiert H, Szopa A. 2023. Health-promoting approaches of the use of chia seeds. Journal of Functional Foods. 103:105480. doi:10.1016/j.jff.2023.105480.

Nozoye T, Aung MS, Masuda H, Nakanishi H, Nishizawa NK. 2017. Bioenergy grass [*Erianthus ravennae*(L.) Beauv.] secretes two members of mugineic acid family phytosiderophores which involved in their tolerance to Fe deficiency. Soil Science and Plant Nutrition. 63(6):543–552. doi:10.1080/00380768.2017.1394168.

Ofori KF, Antoniello S, English MM, Aryee ANA. 2022. Improving nutrition through biofortifica-tion – A systematic review. Frontiers in Nutrition. 9. doi:10.3389/fnut.2022.1043655.

Orona-Tamayo D, Valverde ME, Paredes-López O. 2017. Chia—the new golden seed for the 21st century: nutraceutical properties and technological uses. In: Nadathur S, Wanasundara JPD, Scanlin L, editors. Sustainable Protein Sources. Elsevier; p. 265–281. doi:10.1016/B978-0-12-802778-3.00017-2.

Pestana M, David M, de Varennes A, Abadía J, Faria EA. 2001. Responses Of "Newhall" orange trees to iron deficiency in hydroponics: effects on leaf chlorophyll, photosynthetic efficiency,

and root ferric chelate reductase activity. Journal of Plant Nutrition. 24:1609–1620. doi:10.1081/PLN-100106024.

Pestana M, de Varennes A, Faria EA. 2004. Lime-induced iron chlorosis in fruit trees. In: Dris R, Jain SM, editors. Production practices and quality assessment of food crops. Plant mineral nutrition and pesticide management. Dordrecht: Kluwer Academic Publishers. The Netherlands, ISBN 1-4020-1699-9, p. 171.

Pestana M, Domingos I, Gama F, Dandlen S, Miguel MG, Pinto JL, de Varennes A, Miguel P. 2011. Strawberry recovers from iron chlorosis after foliar application of a grass-clipping extract. Journal of Plant Nutrition and Soil Science. 174:473–479. doi:10.1002/jpln.201000215.

Pestana M, Varennes Ad, Abadía J, Faria EA. 2005. Differential tolerance to iron deficiency of citrus rootstocks grown in nutrient solution. Scientia Horticulturae. 104:25–36. doi:10.1016/j.scienta.2004.07.007.

Rajniak J, Giehl RF, Chang E, Murgia I, von Wirén N, Sattely ES. 2018. Biosynthesis of redox-active metabolites in response to iron deficiency in plants. Nature Chemical Biology. 14 (5):442–450. doi:10.1038/s41589-018-0019-2.

Romera FJ, Alcantara E. 1994. Iron-deficiency stress responses in cucumber (*Cucumis sativus* L.) roots (A possible role for ethylene?). Plant Physiology. 105:1133–1138. doi:10.1104/pp.105.4.1133.

Römheld V. 1987. Different strategies for iron acquisition in higher plants. Physiologia Plantarum. 70:231–234. doi:10.1111/j.1399-3054.1987.tb06137.x.

Saavedra T, Gama F, Aparecida M, Abadía J, de Varennes A, Pestana M, Gonzalez A, Miguel P. 2022. Effects of foliar application of organic acids on strawberry plants. Plant Physiology and Biochemistry. 188:12–20. doi:10.1016/j.plaphy.2022.08.004.

Thimm O, Essigmann B, Kloska S, Altmann T, Buckhout TJ. 2001. Response of arabidopsis to iron deficiency stress as revealed by microarray analysis. Plant Physiology. 127:1030–1043. doi:10.1104/pp.010191.

Tripathi DK, Singh S, Gaur S, Singh S, Yadav V, Liu S, Singh VP, Sharma S, Srivastava P, Prasad SM, et al. 2018. Acquisition and homeostasis of iron in higher plants and their probable role in abiotic stress tolerance. Frontiers in Environmental Science. 5. doi:10.3389/fenvs.2017.00086.

Vasconcelos MW, Clemente TE, Grusak MA. 2014. Evaluation of constitutive iron reductase (*AtFRO2*) expression on mineral accumulation and distribution in soybean (*Glycine max.* L). Frontiers in Plant Science. 5:112. doi:10.3389/fpls.2014.00112.

Vélez-Bermúdez IC, Schmidt W. 2022. Plant strategies to mine iron from alkaline substrates. Plant and Soil. 483:1–25. doi:10.1007/s11104-022-05746-1.

Vélez-Bermúdez IC, Schmidt W. 2023. Iron sensing in plants. Frontiers in Plant Science. 14:1–7. doi:10.3389/fpls.2023.1145510.

Wang D, Datta R, Danish S, Nasim W. 2023. Bio-fortification of two wheat cultivars with iron and zinc through their soil and foliar application in salt-factored soil: growth, ionic, physiological, and biochemical modifications. Journal of Plant Growth Regulation. 42. doi:10.1007/s00344-023-10955-8.

Younis ME, Rizwan M, Tourky SMN. 2021. Assessment of early physiological and biochemical responses in chia (*Salvia hispanica* L.) sprouts under salt stress. Acta Physiologiae Plantarum. 43:121. doi:10.1007/s11738-021-03285-3.

Zuluaga MYA, Cardarelli M, Rouphael Y, Cesco S, Pii Y, Colla G. 2023. Iron nutrition in agriculture: from synthetic chelates to biochelates. Scientia Horticulturae. 312:111833. doi:10.1016/j.scienta.2023.111833.