Journal of Plant Interactions

ISSN: 1742-9145 (Print) 1742-9153 (Online) Journal homepage: https://www.tandfonline.com/loi/tjpi20

Chlorosis correction and agronomic biofortification in field peas through foliar application of iron fertilizers under Fe deficiency

Ahmad Humayan Kabir, Nick Paltridge & James Stangoulis

To cite this article: Ahmad Humayan Kabir, Nick Paltridge & James Stangoulis (2016) Chlorosis correction and agronomic biofortification in field peas through foliar application of iron fertilizers under Fe deficiency, Journal of Plant Interactions, 11:1, 1-4, DOI: 10.1080/17429145.2015.1125534

To link to this article: https://doi.org/10.1080/17429145.2015.1125534

Published online: 12 Jan 2016.

JOURNAL OF PLANT INTERACTIONS, 2016
VOL. 11, NO. 1, 1–4
http://dx.doi.org/10.1080/17429145.2015.1125534

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

OPEN ACCESS

Chlorosis correction and agronomic biofortification in field peas through foliar application of iron fertilizers under Fe deficiency

Ahmad Humayan Kabir[a], Nick Paltridge[b] and James Stangoulis[b]

[a]Plant and Crop Physiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh; [b]School of Biological Sciences, Flinders University, Bedford Park, Australia

ABSTRACT

Effectiveness of different iron (Fe) foliar sprays for leaf chlorosis correction and grain Fe boosting was studied in field peas under Fe deficiency. No chlorophyll reduction was observed in Fe deficient plants treated with foliar sprays. EDDHA [ethylenediamine-*N*,*N*′-bis(2-hydroxyphenylacetic acid)] followed by $FeSO_4$ (73.7 mg/l Fe) treated at the start of flowering was most responsive in correcting chlorosis and increasing shoot dry biomass in peas. Inductively coupled plasma-atomic emission spectroscopy data showed significant increase of Fe in grains while treated with all foliar sprays at the time of grain filling in Fe-deficient plants. Among them, $FeSO_4$ (73.7 mg/l Fe) was the most efficient in biofortifying Fe in mature grain under Fe deficiency in peas. Results also pinpoint that flowering is a suitable time for applying foliar sprays to boost Fe in mature grains. Taken together, application of Fe foliar sprays facilitated both chlorosis correction and Fe boosting in peas and can be further used by breeders and farmers.

ARTICLE HISTORY
Received 27 September 2015
Accepted 24 November 2015

KEYWORDS
Foliar spray; calcareous soil; chlorosis correction; biofortification

Introduction

Iron (Fe) deficiency is a very common problem in calcareous soil and affects numerous agricultural crops including peas throughout the world (Mengel et al. 1982; Moraghan & Mascagni 1991; Welch & Graham 2003). Fe is needed to produce chlorophyll; hence its deficiency causes chlorosis turning yellow or brown in the margins between the veins which may remain green, while young leaves may appear to be bleached (Seeliger & Moss 1976; Haydon & Cobbett 2007; Broadley et al. 2007; Christin et al. 2009). Fe is also essential for plant growth, photosynthesis, enzymatic processes such as those related to oxygen and electron transport, nitrogen fixation, DNA and chlorophyll biosynthesis (Briat 2007; Jeong & Guerinot 2009). Increasing Fe concentration in food crops is an important global challenge due to high incidence of Fe deficiency in human populations. Beside transgenic approaches, enrichment (biofortification) of food crops with Fe through agricultural approaches is a widely applied strategy (Pfeiffer & McClafferty 2007; Borg et al. 2009).

Control of Fe chlorosis is not easy and can be expensive too. Most of the studies dealing with soil and foliar application of Fe fertilizers focused on correction of Fe deficiency chlorosis and improving yield (Rombola et al. 2000). Few studies have been conducted to investigate a role of foliar-applied Fe fertilizers in improving shoot and grain Fe concentration in wheat (Aciksoz et al. 2011) and soybean (Rodriguez-Lucena et al. 2010). Few widely used foliar spray for correcting Fe deficiency are $FeSO_4$, EDDHA [ethylenediamine-*N*,*N*′-bis(2-hydroxyphenylacetic acid)], Fe EDTA (ethylenediaminetetraacetic acid) and Ligno sulfate (Sahua & Singha 1987; Alva & Obreza 1997; Rombola et al. 2000). Lack of consistent results may be related to inconsistent levels of chlorosis severity, soil, environmental and genetic differences. But application of Fe chelates does not represent a sustainable way for the farmers to prevent Fe chlorosis because of the high cost and environmental risks associated with their use (Šramek & Dubsky 2009). EDDHA (Sequestrene 330), which contains 10% Fe, is being used by farmers for correcting Fe deficiency in certain crops in slightly acidic to slightly alkaline soils. However, the high cost of this product is a major limitation though. Another commonly used fertilizer for the correcting Fe chlorosis is ferrous sulfate ($FeSO_4$). Several authors reported that the supplementation of $FeSO_4$ increased grain yield of corn and sorghum grown on Fe-deficient soil (Chad et al. 2003; Patel et al. 2004). Though many Fe sources and methods of application have been tested to correct Fe chlorosis, no effective and complete solution is found yet in peas.

Field pea (*Pisum sativum*) is an important legume and rich in nutritional value. Most of the soil in South Australia is Fe deficient and it is rather a big problem to have good yield of peas in these soils. Therefore, correcting Fe deficiency chlorosis and boosting grain Fe content have become an urgent issue. Thus, the aim of this study was to identify the efficiency of different Fe foliar spray to correct Fe deficiency chlorosis in peas grown in calcareous soil. Furthermore, selection of suitable time for applying foliar spray was also investigated. Another aim of the study was to determine the efficacy of different Fe foliar sprays to boost Fe in mature seeds of field peas grown under Fe deficiency. Taken together, the study was to justify different Fe foliar sprays based on treatment dose, cost and effectiveness for chlorosis correction and agronomic biofortification in peas grown on Fe-deficient soil.

Materials and methods

Plant materials and soil type

Seeds of field peas (var. Parafield) were grown in small pots (one seed per pot) containing 500 g of soil in each pot

CONTACT Ahmad Humayan Kabir ahmad.kabir@ru.ac.bd

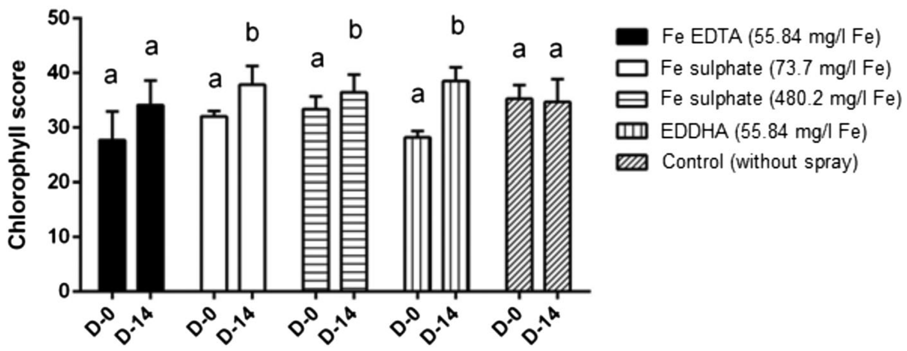

Figure 1. Chlorophyll score in young leaf before and after the foliar spray treatment grown in Fe-deficient soil (start of flowering). D-0 and D-14 represent day 0 and day 14, respectively. Different letters indicate significant differences between means ± SD of treatments ($n = 3$); comparisons were done for D-0 and D-14 conditions.

(Debco, Native mix, Australia) in glasshouse. This soil contained all traces elements and growth stimulants needed for normal growth and development of plants. It also contained controlled release fertilizer and saturaid wetting agent without any native $CaCO_3$. The soil was having no organic compounds and pH (5.5) was suitable for plants loving acidic conditions. Fe deficiency in soil was indirectly induced by mixing 3% $CaCO_3$ with air-dried soil before sowing (Ma et al. 2005; Briat 2007). Addition of $CaCO_3$ increased the pH up to 7.5 that makes the Fe unavailable for plants. Temperature (25°C) and relative humidity (65–75%) were maintained in the glasshouse all through the experiment and proper irrigation was provided in every 2-day interval.

Liquid foliar fertilizers

Different types of liquid foliar fertilizers mostly supplied by Spraygro Australia have been used in this study. These were diluted with water and applied as follows: Fe EDTA (55.84 mg/l Fe), Fe sulfate (73.7 mg/l Fe), Fe sulfate (480.2 mg/l Fe) and EDDHA (55.84 mg/l Fe). Foliar applications are made directly on the leaves (abaxial leaf side) at the starting of flowering and grain filling. Each treatment was applied at a rate of 10 ml/m^2 area and applied twice in one week interval.

Chlorophyll determination

Chlorophyll score was measured in fully expanded young leaves by using SPAD meter (Minotola, Japan) before and after (2 weeks) the treatment of foliar sprays. Data were taken at before applying the foliar spray (day-0) and 14 days (D-14) after applying foliar spray.

Measurement of shoot dry weight

Whole shoot samples were harvested from plants and then dried in an oven at 70°C for 2 days before dry weight was measured.

Determination of Fe concentration in grain

Mature seeds were collected 3 months after sowing and dried in microwave oven at 80°C before grinding 2 g of seed for each sample by using Retsch mill. Mineral analysis was undertaken by inductively coupled plasma-atomic emission spectrometry (ICP-OES) at Waite Analytical Service (WAS), University of Adelaide, Australia. Analysis was done according to WAS Digestion Code = PA. Sample was digested with the mixture of nitric acid (HNO_3) and perchloric acid in tubes followed by heat treatment and resuspension in deionized water according to WAS code and Dahlquist and Knoll (1978). The limit of determination for the sample was calculated as 10× the standard deviation of the calibration blank.

Measurement of shoot dry weight

Shoots of plants were harvested and dried in oven at 70°C for 3 days before measuring in a digital balance.

Statistical analysis

There were three replications for each sample in all experiments conducted in this study. Statistical analyses (t-test) were performed using Genstat software (14th edition). Significance was set at $p \leq .05$.

Results and discussion

Corrections of Fe deficiency chlorosis

It was found that chlorophyll score was unchanged in plants having no foliar spray treatment (Figure 1). However, all Fe foliar sprays found to be efficient in correcting Fe deficiency chlorosis resulting increase chlorophyll score in the subsequent days treated at the start of flowering. Similar results were also found when treated during grain filling though the increase due to Fe sulfate (480.2 mg/l Fe) was not statistically significant. However, the substantial difference in chlorophyll score between control and treatment at day 0 could be associated with the environmental variations of greenhouse. Furthermore, shoot dry weight of whole plants was significantly increased due to foliar application of all sprays when applied at the time of flowering (Table 1). However, shoot dry weight was only significantly increased for EDDHA treated during grain filling (Table 1). Comparatively, EDDHA

Table 1. Shoot dry weight (g/plant) of plants 2 weeks after the foliar spray treatment during start of flowering and grain filling grown in Fe-deficient soil.

Types of foliar spray	Start of flowering	Start of grain filling
Fe EDTA (55.84 mg/l Fe)	$1.93 \pm .04^{ab}$	$4.20 \pm .26^{aa}$
Fe sulfate (73.7 mg/l Fe)	$1.90 \pm .03^{ab}$	$4.40 \pm .10^{aa}$
Fe sulfate (480.2 mg/l Fe)	$1.86 \pm .02^{ab}$	$4.00 \pm .10^{aa}$
EDDHA (55.84 mg/l Fe)	$2.16 \pm .21^{ab}$	$4.66 \pm .05^{ab}$
Control (without spray)	$1.79 \pm .04$	$4.20 \pm .20$

Notes: There were three replications for each sample. Different letters indicate significant differences with control.

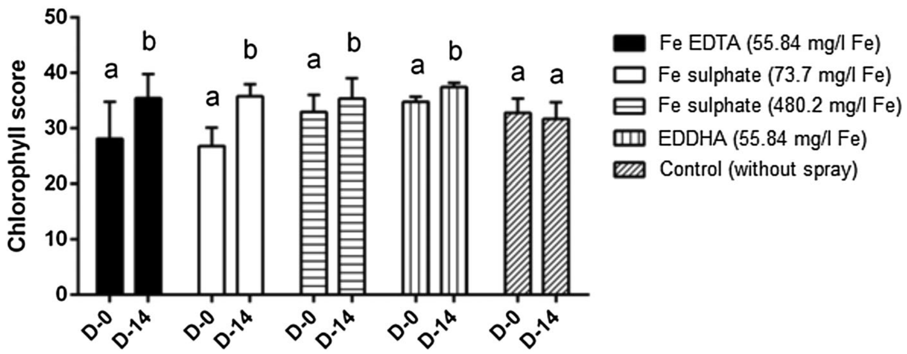

Figure 2. Chlorophyll score in young leaf before and after the foliar spray treatment grown in Fe deficient soil (start of grain filling). D-0 and D-14 represent day 0 and day 14, respectively. Different letters indicate significant differences between means ± SD of treatments ($n = 3$); comparisons were done for D-0 and D-14 conditions.

followed by Fe EDTA were most responsive to increase chlorophyll score and shoot dry weight treated at the start of flowering. It suggests that application of foliar spray prevents Fe deficiency chlorosis and maintains normal physiological growth in peas. Use of EDDHA as foliar spray for reducing chlorosis has been reported in several plants (Alva & Obreza 1997). It was also reported that application of Fe sulfate, elemental sulfur, wettable sulfur and Fe-EDTA decreased chlorosis and increased chlorophyll and carotenoid contents of leaves, uptake of Fe, S and Zn and pod yield of groundnut (Singh et al. 1990). Similarly, foliar application of 0.2% Fe-EDDHA increased chlorophyll a and b and caused marginal increase in nitrogen concentration of plants (Sahua & Singha 1987).

In our study, Fe sulfate (73.7 mg/l Fe) was the most efficient in correcting Fe deficiency chlorosis followed by EDTA and EDDHA treated during grain filling (Figure 2). Efficiency of Fe sulfate is very encouraging since it is cheap that might be of interest to farmers. The effectiveness of Fe sulfate could be due to the functioning of the reductase activity, once the applied Fe(II) has been oxidized to Fe (III). We recommend using Fe sulfate in this stage of plant development for the above mentioned purpose. $FeSO_4$ sprays (0.5%) corrected deficiency symptoms and increased yields by up to 50% in chickpea (*Cicer arietinum* L.) cultivars inefficient in Fe utilization under high pH calcareous conditions (Saxena & Sheldrake 1980). Severe Fe deficiency in peas grown in high pH soil was successfully ameliorated by the application of $FeSO_4$ as foliar spray (Seeliger & Moss 1976; Alvarez-Fernandez et al. 2004; Patel et al. 2004).

It was also interesting from our data that flowering time is more suitable than grain filling time for correcting Fe deficiency in leaves of peas. These results may imply that a successful leaf penetration of Fe could have taken place during grain filling in peas. The superiority of foliar spray depends on the penetration into the tissue, which is a complex process and depends on both environmental and plant factors (Fernandez & Ebert 2005; Astaraei & Ivani 2008).

Boosting of Fe in grains

ICP-OES analysis showed that all foliar sprays applied at the time of flowering were able to boost Fe in the grains when the plants were grown in Fe-deficient soil (Table 2). Fe sulfate (73.7 mg/l Fe), Fe sulfate (480.2 mg/l Fe) and EDDHA were the most efficient to boost Fe in grains applied at the time of flowering even though the plants were grown in Fe-

Table 2. Fe concentration (mg/kg) in mature seeds treated with different foliar sprays grown in Fe-deficient soil.

Types of foliar spray	Start of flowering	Start of grain filling
Fe EDTA (55.84 mg/l Fe)	76 ± .6^{ab}	67 ± 6.8^{aa}
Fe sulfate (73.7 mg/l Fe)	107 ± .5^{ab}	85 ± .7^{aa}
Fe sulfate (480.2 mg/l Fe)	104 ± .8^{ab}	79 ± 7.0^{ab}
EDDHA (55.84 mg/l Fe)	96 ± 3.6^{ab}	72 ± .3^{ab}
Control (without spray)	79 ± 1.9	

Notes: There were three replications for each sample. Different letters indicate significant differences with control.

deficient soil (Table 2). Highest Fe concentration in grains was found by Fe sulfate (73.7 mg/l Fe) treated at the time of flowering. In contrast, foliar sprays used at the time of grain filling were not able to boost Fe in grains under Fe-deficient conditions (Table 2). Singh et al. (1990) reported that application of Fe sulfate and Fe pyrite showed higher Fe and S uptake than other treatments. But EDDHA was found to be less effective in maintaining Fe in seed compared to other foliar sprays used in this study (Table 2). This less efficacy of Fe-EDDHA might be due to high phosphorus concentration in peas and this is also reported in groundnut (Singh et al. 1990). Comparatively, foliar sprays applied during the grain filling was found to be less effective in Fe boosting in grains. Our findings suggest that spraying Fe may represent important agronomic practices to contribute to increasing grain Fe concentrations in peas.

Conclusion

In this study, chlorosis correction and boosting of Fe in grain were successfully demonstrated by the application of foliar sprays. These findings will bring great potential to farmers in both chlorosis prevention and biofortification purposes in peas grown under Fe deficiency. Moreover, these studies will help optimizing Fe spray formulations to make foliar fertilization a reliable strategy in the future to control Fe deficiency in peas. Further research is needed to optimize the process, including chemical composition of the treatments, doses, timing and frequencies.

Acknowledgements

The authors would like to thank SprayGro liquid fertilizers, Australia for supplying the foliar sprays for the research purposes.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Aciksoz SB, Yazici A, Ozturk L, Cakmak I. 2011. Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizers. Plant Soil. 349:215–225.

Alva AK, Obreza TA. 1997. Correction of iron deficiency in orange and grapefruit trees in high pH soils. Proc Fla State Hortic Soc. 110:32–36.

Alvarez-Fernandez A, Garcia-Lavina P, Fidalgo J, Abadia J, Abadia A. 2004. Foliar fertilization to control iron chlorosis in pear (*Pyrus communis* L.) trees. Plant Soil. 263:5–15.

Astaraei AR, Ivani R. 2008. Effect of organic sources as foliar spray and root media on nutrition of cowpea plant. American-Eurasian J Agr Environ Sci. 3:352–356.

Borg S, Brinch-Pedersen H, Tauris B, Holm PB. 2009. Iron transport, deposition and bioavailability in the wheat and barley grain. Plant Soil 325:15–24.

Briat JF. 2007. Iron dynamics in plants. Adv Bot Res. 46:137–180.

Broadley M, White R, Hammond PJ, Zelko JP, Lux I. 2007. Zinc in plants. New Phytol. 173:677–702.

Chad BG, John PS, Alan JS, Randal KT, Curtis RT, Ronald JG. 2003. Correcting iron deficiency in corn with seed row–applied iron sulfate. Agron J. 95:160–166.

Christin H, Petty P, Ouertani K, Burgado S, Lawrence C, Kassem MA. 2009. Influence of iron, potassium, magnesium, and nitrogen deficiencies on the growth and development of sorghum (*Sorghum bicolor* L.) and sunflower (*Helianthus annuus* L.) seedlings. J Biotechnol Res. 1:64–71.

Dahlquist RL, Knoll JW. 1978. Inductively coupled plasma-atomic emission spectrometry: analysis of biological material and soils for major, trace and ultra-trace elements. Appl Spectrosc. 32:1–29.

Fernandez V, Ebert G. 2005. Foliar iron fertilization: a critical review. J Plant Nutr. 28:2113–2124.

Haydon MJ, Cobbett CS. 2007. Transporters of ligands for essential metal ions in plants. New Phytol. 174:499–506.

Jeong J, Guerinot ML. 2009. Homing in on iron homeostasis in plants. Trend Plant Sci. 14:280–285.

Ma C, Tanabe K, Itai A, Tamura F, Chun J, Teng Y. 2005. Tolerance to lime induced iron chlorosis of Asian pear rootstocks (*Pyrus* spp.). J Jpn Soc Hortic Sci. 74(6):419–423.

Mengel K, Bubl W, Scherer HW. 1982. Iron distribution in vine leaves with HCO_3^- induced chlorosis. J Plant Nutr. 7:715–724.

Moraghan JT, Mascagni HJ. 1991. Environmental and soil factors affecting micronutrient deficiencies and toxicities. In: Mortvedt JJ, Fox FR, Shuman LM, Welch RM editors. Micronutrients in agriculture. 2nd ed. Madison, WI: SSSA; p. 371–425.

Patel GJ, Ramakrishnayya BV, Patel BK. 2004. Effect of soil and foliar application of ferrous sulphate and of acidulation of soil on iron chlorosis of paddy seedlings ingoradu soil nurseries in India. Plant Soil 46:209–219.

Pfeiffer WH, McClafferty B. 2007. Biofortification: breeding micronutrient-dense crops. In: Kang MS, Priyadarshan PM, editors. Breeding major food staples. New York: Blackwell Science; p. 61–91.

Rodriguez-Lucena P, Ropero E, Apaolaza-Hernandez L, Lucena JJ. 2010. Iron supply to soybean plants through the foliar application of IDHA/Fe^{3+}: effect of plant nutritional status and adjuvants. J Sci Food Agr. 90:2633–2640.

Rombola AD, Broggemann W, Tagliavini M, Marangoni B, Moog PR. 2000. Iron source affects iron reduction and re-greening of kiwifruit (*Actinidia deliciosa*) leaves. J Plant Nutr. 23:1751–1765.

Saxena MC, Sheldrake AR. 1980. Iron chlorosis in chickpea (*Cicer arietinum* L.) grown on high pH calcareous vertisols. Field Crops Res. 3:211–214.

Sahua MP, Singha HG. 1987. Effect of sulphur on prevention of iron chlorosis and plant composition of groundnut on alkaline calcareous soils. J Agr Sci. 109:73–77.

Seeliger MT, Moss DE. 1976. Correction of iron deficiency in peas by foliar sprays. Aust J Exp Agr Anim Husbandry. 16:758–760.

Singh AL, Joshi YC, Chaudhari V, Zala PV. 1990. Effect of different sources of iron and sulphur on leaf chlorosis, nutrient uptake and yield of groundnut. Fertilizer Res. 24:85–96.

Šramek F, Dubsky M. 2009. Occurrence and correction of chlorosis in young petunia plants. HortScience. 36:147–153.

Welch RM, Graham RD. 2003. Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot. 55:353–364.