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PRELIMINARY STUDIES ON THE EFFECT OF Fe-nanosponge COMPLEX IN HORTICULTURE

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Abstract. Iron deficiency is a major nutritional disorder in crops. Inorganic Fe-compounds and synthetic Fe-chelates are commonly used to control chlorosis but their use arise environmental concerns. We recently developed a new iron fertilizer using a β-cyclodextrin-based nanosponge complex (Fe-NS). In this study, a pilot trial was performed on hydroponically cultivated horticultural plants in order to evaluate the effect of Fe-NS. Sweet corn and tomato were used as model plants analyzing chlorophyll, dry matter and Fe content. Fe-NS effect was compared to $FeSO_4$ and Fe-DTPA. Fe-NS had a positive effect on re-greening and growth in sweet corn and tomato plants.

Key words: iron chlorosis, Fe-fertilizers, nanosponges, sweet corn, tomato

INTRODUCTION

Mineral nutrients are essential for plant growth and development in soil and inert media [Marschner 1995, Chohura and Komosa 2003]. Depending on how great the growth requirement for a given nutrient, the nutrient is referred to as either a macronutrient or a micronutrient. Among the micronutrients, iron is an essential nutrient for plant growth and development, since it participates in life-sustaining processes, such as respiration and photosynthesis [Martínez-Cuenca et al. 2013]. Plants have developed two different strategies in response to Fe shortage: Strategy I, which occurs in dicotyledonous and non-grass monocotyledonous species and Strategy II, which occurs in

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Poaceae species. Strategy I plants can mobilize and take up Fe^{3+} ions from soil particles by acidification of the rhizosphere. Strategy II consists of the roots releasing high affinity chelating compounds (phitosiderophores), which are able to form stable complexes with the micronutrient [Marschner et al. 1986]. In both strategies, Fe deficiency induces several mechanisms aimed to increase Fe uptake from the soil [López-Millán et al. 2009].

Increasing the amount of crop-available Fe has long been carried out by means of iron-fertilizer application to soil and irrigation water. There is a very large number of Fe-contain fertilizers, with different degrees of effectiveness due to many different factors [Abadía at al. 2004]. Iron fertilizers are grouped into three main classes: inorganic Fe-compounds (Fe salts), synthetic Fe-chelates and natural Fe-complexes. Most used fertilizers are synthetic Fe-chelates [Lucena 2007]; this fertilizers are very effective but are expensive and their persistence in soil and groundwater arises environmental concerns [Sánchez-Alcalá et al. 2012]. Due to environmental risks associated with their use, alternative strategies have been suggested [Rombolà et al. 2003, Rombolà and Tagliavini 2007]. In the last years, research on Fe fertilizers has focused on development of new fertilizers, including slow-release and environmentally-friendly [Bhattacharya et al. 2007, Chandra et al. 2009]. Good practices to assess the efficiency of Fe-fertilizers are provided by the current knowledge on plant physiology and biochemistry, which not always taken into account in Fe-fertilizer efficiency agronomical studies [El-Jendoubi 2011].

In this context we developed a new iron fertilizer using β-cyclodextrin-based nanosponge complex (Fe-NS). Cyclodextrin-based nanosponges (NS) are biocompatible nanoporous nanoparticles synthesized by the carbonyl or dicarboxylate hyper-cross-linking of cyclodextrins, as reported in the patent [Trotta and Tumiatti 2003]. The presence of cross-linking and cyclodextrin cavities in their structure favors interaction with active molecules. Different type of molecules, either lipophilic and hydrophilic, may be encapsulated, forming either inclusion or non-inclusion complexes, and carried within the NS structure [Ma and Li 1999]. Recently, nanosponge complexes have been developed to deliver new anti-ethylene compounds to improve the cut flower vase life; and phythormones to improve the regeneration of ornamental plants *in vitro* [Seglie et al. 2008, Seglie et al. 2011, Seglie et al. 2013]. In horticulture NS are relatively unesplored.

The aim of this study was to investigate the effect of Fe-NS in the mineral nutrition of plants. Agronomic trials were performed on hydroponically cultivated horticultural plants. Sweet corn (Strategy II) and tomato (Stategy I) has been selected as model plants [Sharma and Sanwal 1992, Hell and Stephan 2003, Zuchi et al. 2009].

MATERIALS AND METHODS

FE-NS complex synthesis. Fe-NS were developed as iron delivery system. For this purpose, β-cyclodextrins (WeckerChemie) were cross linked with pyromellitic anhydride (Sigma Aldrich) (1 : 5 molar ratio) at 90°C for 2 hours. The β-cyclodextrin was dried at 120°C to constant weight and the pyromellitic anhydride was used without further purification. Dimethyl Sulfoxide (DMSO) was used as solvent (two times the total mass of the reagents) and triethyl amine as catalyst. The compact and dense final product (β-cyclodextrin-based nanosponges, NS), that has the tendency to became a gel in polar solvents, was smashed, purified with acetone (reflux in soxhlet for 12 hours) and milled to a fine powder.

Fe-NS was prepared stirring 18.8 g NS in 100 ml 9.4% $FeSO_4 \times 7H_20$ water solution for 24 h. The solution was preventively acidified with HNO_3 (pH3), to prevent precipitation of Fe hydroxides.

Infrared transmission E.S.P. spectra was performed with a Nicolet Avatar 330 FT-IR through a KBr disc (4% NS w/w). pH stability was assessed by adding 0.1 M HCl and 0.1 M NaOH to a 1.85% (w/w) suspension in distilled water, measuring pH until NS complete dissolution. Thermal stability in air was investigated through a TGA Q50 V20.10 Build 36. instrument.

Considering the stability in the nutrient solution, the Fe-NS synthesis do not generate by products [Roggero et al. 2013].

Agronomic trials. Three separate experiments in hydroponical cultivation were carried out on sweet corn (*Zea mays* L., Challenger F1) and tomato (*Lycopersicum esculentum* Mill., Elegance F1). Seeds were germinated and grown in perlite. Seedlings were then transferred in glass pots contained 0.45 L of Hoagland nutrient solution (one seedling per pot). Plants were hydroponically grown in a growth camber with a photosynthetic flux density at leaf height of 300 µmol $m^{-2}s^{-1}$ photosynthetic active radiation and a 16 h–24°C/8 h–24°C h day/night regime. Preliminary test was conducted on plants using NS + $FeSO_4$ in the nutrient solution (experiment 1). Then, plant fertilization was performed by adding to nutrient solution 75.8 µM Fe as: +$FeSO_4 \times 7H_2O$ (20% Fe), +Fe-DTPA (11% Fe) and +Fe-NS (7% Fe). A randomized experimental design was organized. Each experiment followed a specific protocol according to the different aims and species and was conducted in duplicate, at once. The effect of Fe-NS was evaluated on chlorophyll, dry matter and Fe content. In all experiments, nanosponges (both NS and Fe-NS) were provided at the same concentration.

Experiment 1. NS test on sweet corn. Preliminary NS test on sweet corn was carried out to exclude problems for NS use. A total of 20 sweet corn seedlings, germinated for 12 days, were transplanted to nutrient solution added with 75.8 µM Fe as $FeSO_4$. Then Half plants were cultivated in the presence of (+NS), the others in absence (-NS). Both shoots and roots were postharvest 26 days after sowing.

Experiment 2. Fertilization test on sweet corn. A total of 24 sweet corn seedlings were germinated for 12 days. Then, plant fertilization was performed by adding to nutrient solution 75.8 µM Fe as: +$FeSO_4 \times 7H_2O$ (20% Fe), +Fe-DTPA (11% Fe) and +Fe-NS (7% Fe). Root and shoot dry matter was evaluated (26 days after sowing).

Experiment 3. Fertilization test on tomato. A total of 24 tomato seedlings were germinated for 14 days. Seedlings were grown for more 21 days in a nutrient solution with 37.9 µM $FeSO_4$ to induce Fe-deficiency and then plant fertilization was performed by adding to nutrient solution 75.8 µM Fe as: +$FeSO_4 \times 7H_2O$ (20% Fe), +Fe-DTPA (11% Fe) and +Fe-NS (7% Fe). At the end of the growing period (49 days) dry matter (root and shoot) was measured.

Data collection. During the cultivation period biometric parameters were measured on all plants twice a week. The chlorophyll content and the re-greening effect of the different treatment was evaluated weekly after treatments by SPAD index values of fully expanded young leaves using a portable SPAD-502 meter (Minolta, Osaka, Japan). SPAD provided a sensitive and accurate index of plant response to Fe treatment [Zuchi et al. 2009]. pH level was regularly monitored to prevent increase in alkalinity level. Shoot and root dry weight was assessed at the end of the trial (30 days after sowing) for biomass evaluation. Fe concentration in leaf tissues (dry weight) of sweet corn and tomato plants were determined by ICP-AES after digestion with concentrated HNO_3.

Statistical analysis. In exp. 1 SPAD index values and dry weights were subjected to Tukey's mean comparison test. In exp. 2 and exp. 3 data were subjected to one-way analysis of variance (ANOVA) and the Ryan-Einot-Gabriel-Welsch (F) post hoc test for multiple comparisons ($P < 0.05$). All the data were computed by means of the SPSS statistical package (version 21.0; SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

FE-NS complex synthesis. A new iron fertilizer were obtained using β-cyclodextrin-based nanosponge complex (Fe-NS). Fe-NS resulted to be chemically and physically stable between 3–10 pH range and to air heat until 300°C. FT-IR transmission spectra showed the stretching ester-carbonyl band at 1740 cm^{-1} and the stretching single bond Carbon-Oxygen band at 1200 cm^{-1} [Roggero et al. 2013].

Agronomic trials. Before treatments Fe-deficiency symptoms were observed in plants. Young leaves showed chlorosis and plant growth was compromised. The application of Fe-fertilizers was provided to correct Fe deficiency symptoms and a better plant development, validating the importance of Fe role in plant nutrition [Abadía 2011].

Experiment 1. NS test on sweet corn. Plants cultivated in nutrient solution with $FeSO_4$, added or not with NS, grew similarly and did not show any visual damages. The SPAD index value of (+NS) plants was higher than (-NS) plants (39.44 and 33.49, respectively). No significant differences were observed. Also in terms of dry weight no statistical differences were remarked among treatments (shoot: (+NS) 0.91 g, (-NS) 0.92 g; root: (+NS) 0.91 g, (-NS) 0.92 g).

Experiment 2. Fertilization test on sweet corn. Initially, plants were kept in Fe-deficiency conditions and showed leaf chlorosis (mean SPAD index value 12.03). When Fe-fertilizers were applied, SPAD index value began to increase up to 21.9 (day 14). This type of time-course of leaf SPAD well represents the re-greening and it is comparable to the one observed in other crops [El-Jendoubi 2011]. No significant differences were observed among treatments according to the different Fe compounds (tab. 1). However, different trends were highlighted. Seven days after treatments, the highest increase in SPAD index values was observed in Fe-NS (20.69) while the lowest in $FeSO_4$ (15.32). After 14 days, a further increase for Fe-NS and $FeSO_4$ was registered, while Fe-DTPA decreased. Regarding dry matter values, significant differences were observed among treatments (tab. 1). The dry weight of Fe-NS plants was higher than the one of plants in $FeSO_4$ (shoot and root) and Fe-DTPA (shoot). Statistical differences were remarked also in Fe content. Fe-NS and $FeSO_4$ leaves showed higher Fe content (152 and 149 ppm, respectively) than Fe-DTPA (77 ppm) (tab. 1).

Table 1. SPAD index value (7 and 14 days after treatment), dry matter and Fe content (after 26 days) on sweet corn plants grown with $+FeSO_4$, +Fe-DTPA, +Fe-NS in the nutrient solution

Treatment	SPAD index value		Dry matter (g)		Fe content (ppm)
	7 days	14 days	root	shoot	leaf
$+FeSO_4$	15.32	16.31	0.26 b	0.56 b	149 a
+Fe-DTPA	17.19	14.39	0.47 a	0.52 b	77 b
+Fe-NS	20.69	21.90	0.48 a	1.01 a	152 a
$P^{\dagger}$	ns	ns	*	*	**

† ns, * and **, non significant or significant at $P \leq 0.05$ or $P \leq 0.01$, respectively

Experiment 3. Fertilization test on tomato. The imposition of Fe-deficiency caused visible symptoms of Fe-deficiency (at leaf level: yellowing of full expanded apical leaves (mean SPAD index value 29.98); at root level: proliferation of lateral roots, increase in the diameter of the sub-apical zone and amplified root hair formation) [Tomasi et al. 2009]. When Fe-fertilizers were applied, SPAD index values began to increase. No significant differences were observed among treatments both at 7 and 14 days (tab. 2). On the contrary, significant differences were highlighted in shoot dry matter and Fe content. Fe-NS shoot dry weight was statistically different from Fe-DTPA treatments (1.88 g). No statistical differences were observed in root dry weight. About Fe content Fe-SO_4 and Fe-NS plants and leaves showed higher Fe content than Fe-DTPA (tab. 2).

Table 2. SPAD index value (7 and 14 days after treatment), dry matter and Fe content on tomato plants (after 49 days) grown with $+FeSO_4$, +Fe-DTPA, +Fe-NS in the nutrient solution

Treatment	SPAD index value		Dry matter (g)		Fe content (ppm)
	7 days	14 days	root	shoot	leaf
$+FeSO_4$	40.28	33.48	0.53	1.63 ab	182 a
+Fe-DTPA	40.97	35.29	0.43	1.53 b	120 c
+Fe-NS	41.63	37.70	0.44	1.88 a	170 b
$P^{\dagger}$	ns	ns	ns	*	**

† ns, * and **, non significant or significant at $P \leq 0.05$ or $P \leq 0.01$, respectively

Overall, in the fertilization tests, $FeSO_4$ treatment was effective. Other Researchers demonstrated that the addition of $FeSO_4$ increases grain yield of corn grown on Fe-deficient soil too [Mathers 1970, Hergert et al. 1996]. Under the condition of the experiments $FeSO_4$ probably remained available to the plants and induced a better plant growth and a higher Fe content in leaves. The effectiveness of Fe sulfate may be improved by the addition of organic substrates able to complex the Fe [Rombolà and Tagliavini 2007]. As we observed in Exp.1 +NS could act complexing $FeSO_4$.

Fe-DTPA was less effective than inorganic forms in plant growth and Fe-content and more expensive especially for low-value crops such as corn because treatments often have to be repeated several times during a growing season [Godsey et al. 2003]. Fe-DTPA is a stable complex. In this study Fe was not available for plants even if Fe-fertilizer was much more present in the nutrient solution. Furthermore their use has significant environmental limitations because they have a little residual effect due to their high solubility and eventual leaching [Nowack 2002, Sánchez-Alcalá et al. 2012]. Although the tested species have two different Fe uptake strategies, Fe-NS supplied in the nutrient solution had a positive effect on re-greening, dry matter and Fe-content both in sweet corn and in tomato plants. Moreover Fe-NS ensure a good plant growth probably due to gradually release nutrients and microelements according with plant needs.

CONCLUSIONS

In conclusion, Fe-NS can be considered as an alternative Fe-fertilizer in horticulture. This pilot investigation opens the possibilities of using Fe-NS as new alternative fertilizer in horticulture, although the comprehension of the mechanism of action needs further elucidations. Future commercial use of Fe-NS as fertilizer will also require studies on different NS types to evaluate optimization. The use of NS complexes could also be broadened for transporting other mineral nutrients.

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WSTĘPNE BADANIA DOTYCZĄCE WPŁYWU KOMPLEKSU NANOGĄBKI Fe W OGRODNICTWIE

Streszczenie. Niedobór żelaza jest głównym zaburzeniem odżywczym roślin uprawnych. Nieorganiczne związki Fe oraz syntetyczne chelaty Fe są powszechnie używane do walki z chlorozą, co niepokoi jednak ekologów. Opracowano więc nowy nawóz żelazowy przy użyciu kompleksu nanogąbki w oparciu o β-cyklodekstrynę (Fe-NS). W niniejszym badaniu przeprowadzono test pilotażowy na roślinach ogrodniczych w celu oceny wpływu Fe-NS. Modelowymi roślinami były słodka kukurydza i pomidor, w których przeanalizowano zawartość chlorofilu, suchej masy oraz Fe. Wpływ Fe-SN porównano z $FeSO_4$ i Fe-DTPA. Fe-NS miał pozytywny wpływ na nowe zazielenienie oraz wzrost roślin słodkiej kukurydzy i pomidora.

Słowa kluczowe: chloroza żelazowa, nawozy Fe, nanogąbki, słodka kukurydza, pomidor

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