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Mineral elements bioavailability in the halophyte species *Suaeda fruticosa*

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The aim of this work was to investigate the morphological and physiological responses of *Suaeda fruticosa* to Fe deficiency. For this purpose, seedlings of *Suaeda fruticosa* were grown under controlled conditions in the presence of iron sufficient (C) or deficient (D) mediums. Plant growth, chlorophyll content, Fe status, rhizosphere acidification and Fe(III)-chelate-reductase activity (FCR) were studied, along with potassium, zinc and copper concentration. Our results showed that *Suaeda fruticosa* shoot and whole plant biomass production were significantly decreased under Fe deficiency conditions, whereas that of roots was not affected by such constraint when compared to the control. Moreover, Fe deficiency resulted in a significant reduction of chlorophyll and Fe concentration. Interestingly, although grown under Fe deficiency conditions, *S. fruticosa* plant was able to increase their shoot iron use efficiency (FeUE), to stimulate acidification capacity and FCR activity in their roots. Furthermore, Fe deficiency led to significant decrease of potassium concentration in *S. fruticosa* roots, while an accumulation of copper was noted. On the contrary, no significant accumulation of zinc was observed. These data suggest that the capacity of *S. fruticosa* plants to maintain plant growth and to preserve adequate chlorophyll synthesis under iron-limiting conditions is related to its better Fe-use efficiency, in addition to its high acidification and root reducing capacities. This allows us to suggest this species is relatively effective in overcoming Fe deficiency.

Key words: Halophytes, iron deficiency, acidification capacity, FCR activity, mineral elements.

Abbreviations: DW: dry weight; FCR: Fe(III)-chelate-reductase; FeUE: iron use efficiency.

INTRODUCTION

Tunisian Chotts and sebkhas are colonized by wild vegetation called halophytes which survive at extreme salinity and mineral deficiencies. Among the nutrient deficiency the most studied, is the topic of iron deficiency (Sleimi & Abdelly, 2001). The Soliman sebkha is a type of saline sodic soil. The exploration of those areas showed the existence of native halophytes vegetation belonging mainly to the family of Chenopodiaceae. For instance, *Suaeda fruticosa* is a perennial succulent halophyte known by its ability to support high levels of salinity and it is used in forage (Khan *et al*., 2000; Sleimi & Abdelly, 2001). When grown in saline and sodic soils, halophytes are subjected to iron acquisition difficulties because in these areas iron solubility is very low, as it is present as oxide and hydroxide compounds inaccessible to plants (Römheld & Marschner, 1986).

It is well known that Fe deficiency alters the morphology and physiology of plants (Briat, 2007); it especially leads to Fe chlorosis resulting from a large decrease in leaf chlorophyll concentration, due to insufficient availability of iron in the soil or difficulties in the uptake of this nutrient by roots (Pestena *et al*., 2005). Against poor solubility of Fe in the soil, plants have evolved a variety of mechanisms to increase Fe mobility and its uptake in the cytosol (Zocchi *et al*., 2007). Indeed, plants developed different strategies allowing them to belong to Strategy I or Strategy II. The first class includes dicotyledonous and non-

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graminous monocotyledonous. This strategy consists in developing a strong ferric reduction activity at the root surface (Chaney *et al.*, 1972); moreover, these plants can induce an active proton extrusion by increasing the H^+-ATPase activity in root plasmalemma leading to the rhizosphere acidification (Zocchi & Cocucci, 1990). The second class is based on the excretion of phytosiderophores in the medium in order to increase iron acquisition.

On the other hand, Fe deficiency was shown to stimulate influx of Fe and other divalent cations in many plant species (Cohen *et al.*, 2004). For instance, Fox *et al.* (1996) found that Fe-deficient pea seedlings exhibited higher rates of Fe influx compared to Fe-sufficient ones. Furthermore, tissue concentrations of Fe and other mineral elements can be influenced by plant Fe status. Welch *et al.* (1993) demonstrated that the shoot concentrations of several divalent cationic metals, including Cu, Mn, and Mg, increased in Fe-deficient pea seedlings compared to Fe sufficient ones. Rodecap *et al.* (1994) also reported that Fe-deficient *Arabidopsis* plants accumulated higher concentrations of Cd and Mg in racemes and seeds compared to Fe-sufficient plants.

In the literature, many works were focused on the effect of Fe deficiency on the physiological and biochemical responses of glycophyte species (M'sehli *et al.*, 2008; Donnini *et al.*, 2009; Jelali *et al.*, 2010). However, as far as we know, no information is available concerning the response of an halophyte species to such constraint. The aim of the present work was therefore to investigate the effect of Fe deficiency on the plant growth, chlorophyll content, in addition to the root acidification capacity and Fe(III)-chelate-reductase (FCR) activity in *Suaeda fruticosa* plants, an halophyte specie abundant in saline ecosystems in Tunisia. In addition, we tried to examine the interaction between Fe, Zn, and Cu in all plant organs.

MATERIALS AND METHODS

Plant material and growth conditions

Suaeda fruticosa seedlings were obtained from the sebkha of Soliman and then transferred to the laboratory to be cultivated in a half strength aerated nutrient solution for 7 days. After that, similar sized seedlings were selected and cultured as groups of 5 plants per container of 5 L in full strength aerated nutrient solution. Two types of treatments were applied for 30 days as follows: sufficient plants or control cultivated in the presence of Fe at 30 μM (Control; C) and deficient ones grown in the absence of Fe (-Fe). Each treatment contained 20 plants (4 replicates of 5 plants per container). The composition of the nutrient solution was: 1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, 0.25 mM KH_2PO_4 and 10 μM H_3BO_3, 1 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.05 μM $(NH_4)_6Mo_7O_{24}$ and 0.4 μM $CuSO_4$. Iron was supplied in the form of Fe(III)-EDTA. Aerated hydroponic cultures were maintained in a growth chamber with a day/night regime of 16/8 hrs, 24 AC/18 AC regime, *PPFD* of 200 μmol m^{-2} s^{-1} at the plant level and a relative humidity of 70%. The solution was renewed weekly. The pH was adjusted to 6.0 with NaOH for both (Control, -Fe) treatments. It is worth mentioning that the plants used for the determination of all the parameters measured (chlorophyll content, plant biomass production, Fe content, FCR and nutrient analysis) were randomly selected from the different containers of each treatment.

Chlorosis index

The chlorosis index was determined in Fe-deficient plants after 30 days of treatment following the scale of Gildersleeve & Ocumpaugh (1989): (0) green young leaves, (1) slight chlorosis with specific yellow leaf margins, (2) yellow limb with green vascular bundles, (3) completely yellow leaves, and (4) severe chlorosis with necrosis symptoms. A number was attributed to each plant according to the chlorotic state of its young leaves and, a mean of 20 replicates was calculated for each treatment.

Chlorophyll concentration

The chlorophyll concentration (mg g^{-1} FW) was determined according to the method of Torrecilas *et al.* (1984). The sub-apical leaves (1 to 2 leaves per plant) were harvested, immediately weighed, crossed in discs, and then used for the extraction of chlorophyll assessment. Five ml of 80% acetone were added to fresh leaf samples (approximately 100 mg). The total extraction took place after 72 hrs in darkness, at 4°C. The extract absorbance was measured at 649 and 665 nm.

Nutrient analysis

At harvest, plants were separated into shoots and roots. Roots were carefully washed once with 1% (v/v) HCl in order to remove extracellular Fe and

then twice with distilled water. After drying at 60°C for 72 hrs, samples were weighted for biomass determination then ground with an agate grinder and digested with a nitric/perchloric acid solution (2.5:1, v/v) for nutrient extraction according to the method of Grusak (1995). Samples were analyzed for micronutrient (Fe, Zn and Cu) in addition to macronutrient by means of a Perkin-Elmer Analyst 100 Atomic Absorption Spectrophotometer (VARIAN 220 FS).

Fe use-efficiency

Shoot Fe-use efficiency was calculated based on the ratio of shoot biomass (mg) to shoot Fe concentration (μg Fe).

Root acidification

During the final 10 days, the pH of nutrient solution of each container was measured every two days with a Metrohm 663 pH-meter. For both (C and -Fe) treatments, the pH of the nutrient solution used for renovation was adjusted to the corresponding value of the old solution.

Fe(III)-chelate-reductase (FCR) activity

Root Fe(III)-chelate reductase (FCR) activity was measured using bathophenantrolinedisulfonate (BPDS) according to the method of Chaney *et al*. (1972). Apical root segments (about 2.5 cm long) were excised from each of five plants randomly selected per treatment. Root segments were incubated in the dark at 25°C under shaking, in 5 ml of a solution with the following composition: 0.5 mM $CaSO_4$, 0.1 mM Fe(III)-citrate, 0.3 mM BPDS, 10 mM 2-(N-morpholin) ethanesulfonic (MES)-NaOH, pH 6.0. After a kinetic survey of 150 min, the absorbance of the solution was determined with a spectrophotometer at 535 nm, and the amount of the reduced Fe(III) was calculated by the concentration of the formed Fe(II)$BPDS_3$ complex, using an extinction coefficient of 22.4 mM^{-1} cm^{-1}. Blank controls without apical root segments were also used in order to correct any unspecific Fe reduction.

Statistical analysis

Variance analysis of data (ONE-WAY ANOVA) was performed using the SPSS 10.0 program. Means were compared using the Duncan's test at $p < 0.05$ when significant differences were found. Data shown are means of five for FCR activity, eight (for plant dry weight, chlorophyll and nutrient contents) and twenty (for acidification capacity) replicates for each treatment.

RESULTS

Chlorosis index and chlorophyll status

At the end of the experimental period, the chlorosis index reached 0.6 in Fe deficient plants of *Suaeda fruticosa* (Table 1). These symptoms were accompanied by a significant decrease in chlorophyll concentration. In fact, Fe deficient plants exhibited a diminution of 55.9%, compared to the control (Table 1).

Plant growth

Although grown under Fe-deficient conditions for 30 days, *Suaeda fruticosa* root DW was not affected by such constraint (Table 1). On the contrary, shoot and whole plant dry weight (DW) were significantly decreased (*ca* 28 and 24%, respectively) (Table 1).

Iron status

Iron concentration was remarkably greater in the control than in Fe-deficient of *S. fruticosa* (Table 2). In addition, a significant decline of Fe concentration

TABLE 1. Chlorosis score, chlorophyll concentration and plant dry weight (DW) in shoots and roots of *S. fruticosa* plants grown for one month under iron-sufficient (Control) or iron-deficient medium (-Fe)

Treatments	Control	-Fe
Chlorosis score	0.00 ± 0.00^b	0.60 ± 0.10^a
Chlorophyll concentration (mg g^{-1} FW)	1.18 ± 0.10^a	0.52 ± 0.05^b
Shoot DW (g $plant^{-1}$)	1.42 ± 0.12^a	$1.02 \pm 0.\ 21^b$
Root DW (g $plant^{-1}$)	0.33 ± 0.08^a	0.30 ± 0.04^a
Plant DW (g $plant^{-1}$)	1.75 ± 0.20^a	1.32 ± 0.15^b

Values are means ± s.e. and differences between means were compared using Duncan's test ($p = 0.05$)

TABLE 2. Iron concentration and content in shoots and roots, iron absorption (FeAE) and shoot use efficiencies (FeUE) of *S. fruticosa* plants grown for one month under iron-sufficient (Control) or iron-deficient medium (-Fe)

Treatments	Control	-Fe
Shoot iron concentration (μg g^{-1} DW)	276.35 ± 42.90^{a}	226.81 ± 18.48^{b}
Shoot iron content (μg $plant^{-1}$)	214.71 ± 55.94^{a}	96.94 ± 40.22^{b}
Root iron concentration (μg g^{-1} DW)	568.19 ± 115.28^{a}	423.82 ± 33.23^{b}
Root iron content (μg $plant^{-1}$)	272.45 ± 8.72^{a}	178.85 ± 40.22^{b}
FeUE (mg DW $μg^{-1}$ Fe)	3.47 ± 0.31^{b}	8.36 ± 0.84^{a}

Values are means ± s.e. and differences between means were compared using Duncan's test ($p = 0.05$)

TABLE 3. Potassium concentration and content in shoots and roots and potassium absorption efficiency (KAE) of *S. fruticosa* plants grown for one month under iron-sufficient (Control) or iron-deficient medium (-Fe)

Treatments	Control	-Fe
Shoot potassium Concentration (mg g^{-1} DW)	61.21 ± 10.28^{a}	69.06 ± 3.99^{a}
Shoot potassium content (mg g^{-1} DW)	58.09 ± 2.41^{a}	51.31 ± 1.20^{b}
Root potassium Concentration (mg g^{-1} DW)	30.00 ± 3.10^{a}	19.22 ± 1.20^{b}
Root potassium content (mg g^{-1} DW)	12.27 ± 2.96^{a}	3.95 ± 0.74^{b}

Values are means ± s.e. and differences between means were compared using Duncan's test ($p = 0.05$)

TABLE 4. Zinc concentration and content in shoots and roots of *S. fruticosa* plants grown for one month under iron-sufficient (Control) or iron-deficient medium (-Fe)

Treatment	Control	-Fe
Shoot zinc concentration (μg g^{-1} DW)	25. 21 ± 6.50^{a}	27.12 ± 6.31^{a}
Shoot zinc content (μg $plant^{-1}$)	28.25 ± 2.66^{a}	25.74 ± 4.70^{a}
Root zinc concentration (μg g^{-1} DW)	26.42 ± 4.22^{a}	23.51± 6.03^{a}
Root zinc content (μg $plant^{-1}$)	12.34 ±1.99^{a}	4.89 ±1.01^{b}

Values are means ± s.e. and differences between means were compared using Duncan's test ($p = 0.05$)

TABLE 5. Copper concentration and content in shoots and roots of *S. fruticosa* plants grown for one month under iron-sufficient (Control) or iron-deficient medium (-Fe)

Treatments	Control	-Fe
Shoot copper concentration (μg g^{-1} DW)	4.34 ± 0.47^{b}	7.67 ± 1.63^{a}
Shoot copper content (μg $plant^{-1}$)	3.45 ± 0.69^{b}	7.27 ± 137^{a}
Root copper concentration (μg g^{-1} DW)	19.73 ± 6.55^{b}	73.82 ± 6.84^{a}
Root copper content (μg $plant^{-1}$)	7.09 ± 0.97^{b}	14.69±4.13^{a}

Values are means ± s.e. and differences between means were compared using Duncan's test ($p = 0.05$)

in shoots and roots was noted under Fe starvation conditions with a sharpest decrease observed in the latter organs. Indeed, the reduction rate was about 17.9 and 25.4%, in shoots and roots, respectively. Content of this nutrient was also decreased, especially in shoots (*ca* 54.8%). Under such severe conditions, *S. fruticosa* plants increased their shoot iron use efficiency (FeUE), reaching 240% of the control (Table 2).

Potassium concentration (K^+)

Potassium concentration was evaluated in shoots and roots of Fe sufficient and Fe deficient *S. fruticosa* plants (Table 3). Under iron deficiency conditions, no significant effect was observed in shoots K^+ concentration, while a significant decrease was detected in roots (*ca* 67.8%) (Table 3). Potassium content was reduced both in shoots and roots.

Zinc (Zn)

As shown in Table 4, no significant accumulation was detected for Zn concentration and content in *S. fruticosa* shoots grown under Fe deficiency conditions, as compared to the control. The same trend was observed in Zn roots concentration. However, a significant decrease was recorded in Zn content (*ca* 60.3%) (Table 4).

Copper (Cu)

Concerning copper analysis, our results showed that Fe deficiency resulted in a significant accumulation of this nutrient concentration in shoots and roots (Table 5). In fact, a significant increase of *S. fruticosa* shoots concentration and content was observed (0.7-fold and 1.1-fold increase, respectively), as compared to the control. Nevertheless, it is important to note that Cu accumulation was greater in *S. fruticosa* roots (2.7-fold increase, for Cu concentration) (Table 5).

Root acidification capacity

The pH of the nutrient solution was measured every two days during the last ten days of treatment and values were represented in Figure 1. In control plants, the medium pH values, initially adjusted to pH = 6, tended to increase and exceeded 7. However, an important acidification of the nutrient solution was observed in Fe deficient roots of *S. fruticosa* plants. Indeed, deficient plants showed the lowest pH value after 30 days of Fe deficiency reaching to 4 pH unit (Fig. 1).

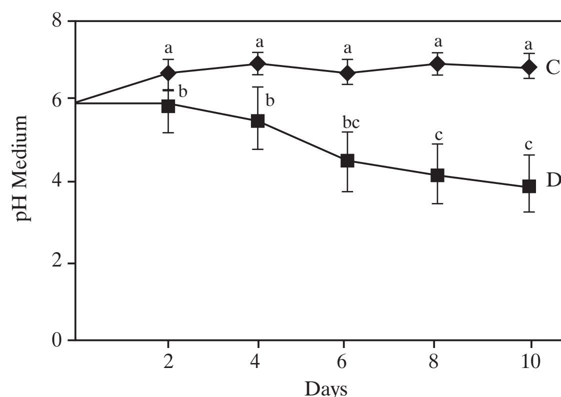

FIG. 1. The pH values of the culture media of *S. fruticosa* plants grown during one month on iron-sufficient (Control), or iron-deficient medium (-Fe). Values are means of five replicates (± standard error). Different letters denote significant different values at $p < 0.05$ according to Duncan's test.

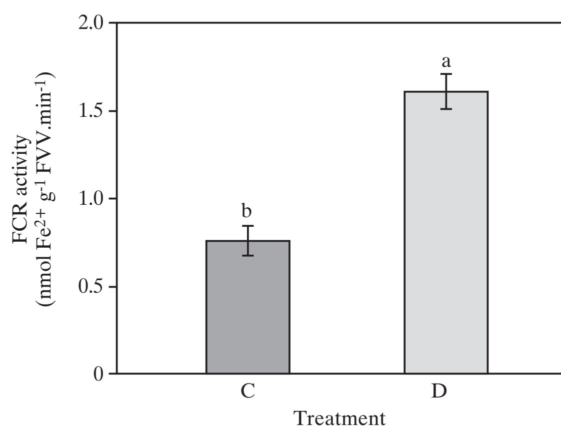

FIG. 2. Root Fe(III)-chelate-reductase (FCR) measured in leaf discs of *S. fruticosa* plants grown during one month on iron-sufficient (Control), or iron-deficient medium (-Fe). Values are means of twenty replicates (± standard error). Different letters denote significant different values at $p < 0.05$ according to Duncan's test.

Fe (III) chelate reductase activity in roots

Fe(III)-chelate-reductase (FCR) activity was measured either in iron sufficient (C) or iron deficient *S. fruticosa* plants (D). Our results showed that FCR activity of root segments increased in Fe deficient plants. Indeed, at the last day of the treatment, FCR activity was enhanced compared to the control (1.1-fold increase) (Fig. 2).

DISCUSSION

As shown above in our results, chlorophyll concentration of *S. fruticosa* leaves was significantly affected

by Fe deficiency (Table 1). For instance, Fe deficiency resulted in a significant reduction of chlorophyll (a and b) and carotenoid contents in pea leaves after 5-7 days of treatment (Iturbe-Ormaetxe *et al*., 1995; Jelali *et al*., 2011). Fe shortage was also shown to decrease chlorophyll concentration in other species such as soybean (Zocchi *et al*., 2007) and medicago (Rabhi *et al*., 2007). Indeed, iron is a necessary micronutrient for chlorophyll biosynthesis as well as thylakoid and granum formation (Marschner & Römheld, 1994). Consequently, iron chlorosis is due to chlorophyll dilution when leaves continue to grow at a normal rate under iron deficiency conditions (Abadía *et al*., 2000) and to cell inability to produce and/or to stabilize new chlorophyll molecules in thylakoid membrane (Belkhodja *et al*., 1998). On the other hand, leaf chlorosis could be the consequence of a necessary bivalent iron deficit for the biosynthesis of chlorophylls (Nikolic & Kastori, 2000), which was also found in Fe deficient plants of *S. fruticosa* (data not shown).

Fe deficiency has been generally shown to adversely affect the growth activity in many plant species (De la Guardia & Alcantara, 2002). In our case, shoot and whole plant DW was significantly reduced by Fe deficiency (Table 2). However, *S. fruticosa* roots have the capacity to maintain their growth. This behaviour is described for tolerant genotypes to Fe deficiency conditions (Marschner *et al*., 1996; Pestana *et al.*, 2005), and often associated with an increased number of secondary and tertiary roots leading to a more efficient exploration of the soil (Bavaresco *et al*., 1994). Interestingly, *S. fruticosa* plants grown under Fe deficiency conditions increased their shoot Fe-use efficiency in comparison to the control (Table 2), which allows this species to maintain plant growth. Therefore, based on root biomass production and Fe-use efficiency (FeUE), we suggest that *S. fruticosa* is relatively tolerant to Fe chlorosis.

In addition to the effect of Fe deficiency on the plant growth and Fe status, several other biochemical mechanisms such as root acidification capacity and Fe reduction mechanism, remain of major importance (Marschner *et al.*, 1996). With regard to the root acidification capacity, our results showed that Fe deficient *S. fruticosa* roots showed an important capacity to decrease the pH of the nutrient solution (Fig. 1). In other species, the ability to lower the pH of the nutrient solution has been suggested as a useful trait for screening tolerant genotypes to Fe deficiency (Dell'Orto *et al*., 2000). In this context, the same authors reported that this proton release occurred via the activation of the plasma membrane proton pump (H^+-ATPase) of root cortical cells is one of the important biochemical mechanisms by which Strategy I plants increase their capacity to mobilize and acquire Fe from the insoluble forms in the soils. Furthermore, the ability of *S. fruticosa* roots to acidify their medium was correlated with the induction of the FCR activity that was stimulated compared to the control (Fig. 2). Thus, these data strongly support that root FCR activity plays an important role in the adaptive response of *S. fruticosa* plants to Fe deficiency. In other species, the increase of this enzyme activity was correlated to the tolerance to Fe deficiency, as it was reported for kiwifruit (Rombolà *et al*., 2002), peach (Molassiotis *et al*., 2006), medicago (M'sehli *et al*., 2008) and pea (Jelali *et al*., 2010). Taken together, the increase observed in the acidification capacity and FCR activity of *S. fruticosa* root segments confirms the relative tolerance of this plant in overcoming this nutritional constraint.

Fe starvation has been generally shown to affect mineral element homeostasis (Welch *et al*., 1993). As shown above in our results, K^+ concentration and content were reduced in Fe deficient roots of *S. fruticosa*. Indeed, Fe deficiency is often associated with K^+ starvation, although some authors suppose that chlorotic leaves frequently reduce K^+ concentration (Loué, 1993; Jelali *et al*., 2011). In this context, Rabhi *et al.* (2007) showed a decrease in K^+ acquisition of medicago plants subjected to Fe deficiency. In addition, our results showed no significant effect of Fe deficiency on the Zn concentration in all *S. fruticosa* plant organs. This result can be explained by the fact that Zn and Fe elements have the same transporters. With this regard, Lombi *et al.* (2002) reported that the transporters of heavy metals have not any specificity and therefore they are able to assimilate any trace element. On the contrary, Cohen *et al.* (1998) showed that Fe deficiency induces an increased capacity to absorb Fe and other micronutrient and heavy metals such as Zn and Cd into pea roots.

Under Fe-deficient conditions, the decrease of Fe concentration was accompanied by an increase in Cu concentration. The antagonism between Fe and Cu was found by McBride *et al.* (2001) in maize plants grown in Cu contaminated soils and by Chen *et al.* (2004) in *Commelina communis*. Furthermore, it was demonstrated that independently of the Cu concentration in the soil, the highest concentration of this element was also observed in maize (Brun *et al*., 2001)

and tomato (Chaignon *et al*., 2003) roots. Consequently, these plants appeared to be able to restrict the translocation of Cu to their aerial parts. This suggests that root accumulate Cu more than shoot and it might be regarded as a barrier, protecting the leaves from toxic levels of Cu. According to Nenova & Stoyanov (1999), the increased concentrations of all nutrients in chlorotic leaves can be explained partially by concentration effect due to restricted growth. However, in our case, the existence of significant rise of Cu concentration within deficient plants allows us suggesting changes on this metal absorption.

In conclusion, the present work clarified some morphological and physiological responses of a perennial halophyte *S. fruticosa* to direct iron deficiency. Based upon the greater ability of this species to maintain plant growth, to acidify efficiently the external medium and to induce the root reducing capacity, leading to more efficient Fe mobilization, we could conclude that *S. fruticosa* proved to be relatively tolerant to Fe deficiency.

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