Article

Mineral nutrition of bean lines under iron chlorosis

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Abstract

Iron deficiency exists in almost every crop in the world and the ability to absorb iron varies widely between plant species. There are groups of plants that are characterized by their ability to grow in soils with low iron availability, called Fe-efficient. In this experiment, bean plants were grown in a greenhouse, in a nutrient solution with suboptimal and optimal concentrations of Fe. The objective was to evaluate the mechanisms of tolerance, concentration and distribution of Fe in bean lines. Six bean lines (three tolerant and three susceptible to iron deficiency) were established. The nutritional concentration and SPAD units were evaluated in young leaves and roots, root volume and dry matter. The results obtained determined a high nutritional imbalance index (NII), transfer coefficient, the ratios P/Fe and K/Ca, concentration of K, Ca, Mg, Mn, Zn, Cu and B in young bean leaves in leaves with iron chlorosis. In the absence of Fe, line 496 showed less chlorosis, the P/Mg ratio and the concentration of P and K increased. When Fe was present in the nutrient solution, lines 496 and 33 had low nutritional indices and higher dry matter production. Line T_2 was susceptible to iron chlorosis, but with a concentration of 1 mg L^{-1} of Fe in the nutrient solution, it had greater production of dry matter, root volume and did not manifest iron chlorosis. Line 33 was susceptible and in the absence of Fe in the solution, the ratios N/P, B/P, Ca/P increased, and the concentration of P, K and B decreased. The addition of 1 mg L^{-1} of Fe in the nutrient solution increased the concentration of N, P, K and Fe, while in the absence, the concentration of Mn, Zn and Cu in root increased. The differences found in iron bean chlorosis in tolerant and susceptible plants are not due to the concentration of Fe but to internal mechanisms, related to other mineral elements that affect their metabolism.

Keywords: iron deficiency, iron stress, plant nutrition, strategy I, strategy II.

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Introduction

Iron (Fe) is an element that is abundant in the earth's crust (Frey *et al*., 2012) and is essential for plants, although its availability is insufficient to keep optimal growth. It is a nutrient that participates in photosynthesis, respiration, DNA synthesis (Rout *et al*., 2015), component of the electron transport chain, enzyme cofactor and chlorophyll synthesis (Schmidt *et al*., 2020), due to its ability to donate and accept electrons (Parrilla *et al*., 2019). Plants are the primary source of Fe for animals and humans, which causes two-thirds of the world's population to be at risk of Fe deficiency anemia (Buccio *et al*., 2004).

Its solubility and availability to plants is low under aerobic conditions, particularly at a high pH and in calcareous soils (Tripathi *et al*., 2018). Although Fe is in high concentrations in the primary minerals of the soil, it undergoes oxidation and precipitates as compounds of low solubility, limiting its availability to plants (Mielki *et al*., 2016).

Plants absorb Fe from the soil through two strategies (Tripathi *et al*., 2018), which are known as strategy I or reducing strategy and strategy II or chelating strategy. The difference between them lies in the oxidation state of Fe when it is absorbed by the plant: Fe^{2+} for strategy I and Fe^{3+} for strategy II (Connorton *et al*., 2017). Strategy I consists of exuding H^+, decreasing the pH of the soil by extrusion of H^+ to increase the solubility of Fe^{3+}, by means of ATPases, then there is a reduction of Fe^{3+} to Fe^{2+} on the surface of the root by a ferric-chelate reductase bound to the plasma membrane and finally an absorption of Fe^{2+} in root cells by the high-affinity Fe transporter AtIRT1 (Wairich *et al*., 2019).

In strategy II, plants secrete phytosiderophores (deoxymugineic acid) with high affinity for Fe. Fe^{3+}-phytosiderophores are imported by the oligopeptide transporter YS1 (Connorton *et al*., 2017). Fe deficiency causes iron chlorosis in plants that grow in calcareous soils (López-Millán *et al*., 2013), which is an important limitation for plant development that affects crop yield and quality (Li *et al*., 2017).

Fe deficiency causes chloroplast degradation and decrease in chlorophyll synthesis, also decreases the fresh weight and photosynthetic rate of the plant (Li *et al*., 2021). Therefore, the objective of the present research was to determine the bean varieties sensitive to iron chlorosis and its influence on the concentration and distribution of nutrients in plant tissue.

Materials and methods

The experiment was carried out in a greenhouse with natural light, where seeds of 6 bean lines (three tolerant and three susceptible) were germinated in trays with vermiculite as a substrate. Bean seedlings with equal vigor, developed up to the first trifoliate leaf, were used. Steiner's Universal solution was used, consisting of 9 me L^{-1} Ca $(NO_3)_2.4H_2O$, 3 me KNO_3, 3 me L^{-1} K_2SO_4, 1 me L^{-1} KH_2PO_4, 4 me L^{-1} $MgSO_4$, 9 µM L^{-1} $MnSO_4.3H_2O$, 45 µM L^{-1} H_3BO_3, 0.3 µM L^{-1} $CuSO_4$, 0.7 µM L^{-1} and $ZnSO_4.7H_2O$, with and without 18 µM L^{-1} or 1 mg L^{-1} Fe-EDDHA.

The treatment design was the combination of the factors: 1) concentration of Fe-EDDHA (0 and 1 mg L^{-1}), pH (6 and 8.5) and 3) bean genotype (6). The experimental design was in randomized complete blocks, with four repetitions. The experimental unit was a pot, which contained 2 plants.

The seedlings were transplanted into 3 L pots with nutrient solution, aerating every hour for 15 minutes for 33 days. The nutrient solution was changed every seven days and the pH was adjusted every three days. The variables evaluated were nutritional concentration (young leaves and root), SPAD readings, root volume and dry matter.

In the nutritional concentration, samples of young leaves and clean roots, without physical, chemical or biological damage, were taken. The samples were placed in paper bags. Each sample was washed and dried at 70 ºC for 48 h in an oven with forced air circulation before being ground (mesh 20) in a stainless-steel mill. The digestion of the material was carried out with a diacid mixture (4 ml HNO_3:2 ml $HClO_4$) at 203 ºC. In the digestate of the plant material, P, K, Ca, Mg, Fe, Mn, Zn, Cu and B were determined by ICP-AES spectrophotometry in a Varian, Liberty II equipment. For N, the digestion of the material was carried out with a mixture of salicylic and sulfuric acid, it was determined by the Kjeldahl method (NOM-021-RECNAT-2000, 2000).

The nutritional ratios (N/P, P/Mg, Cu/P, B/P, Ca/P, Mn/Ca, B/Mg, Mn/Fe, P/Fe, K/Ca, K/Fe, Cu/Fe, Zn/Fe) were obtained with the results of the chemical analysis. SPAD readings were obtained using the Minolta SPAD-502 portable chlorophyll reader. This analytical instrument provides a quantitative estimate of the green color of the leaf, which represents an advantage as it replaces visual scales. For dry matter, the plant was cut into two parts (root and aerial part), and these were placed in the oven at 70 ºC for 48 h with forced air circulation and then weighed. Data were analyzed using the statistical program SAS V8 by comparison between means with the Tukey test (α= 0.05).

Results and discussion

SPAD readings

Figure 1 shows the SPAD readings of the interaction of line and Fe in the solution. When Fe was not added to the solution, line 496 had higher SPAD readings than lines 33 and T_2. The highest SPAD reading without addition of Fe corresponded to line T_2, statistically similar to line 496 but higher than line 33. This shows that line 496 has a better ability to adapt to iron chlorosis, while line T_2 responds favorably only when Fe is present in the nutrient solution, but negatively when it is absent. Line 33 has the highest degree of susceptibility of all lines studied, both in presence and absence of Fe.

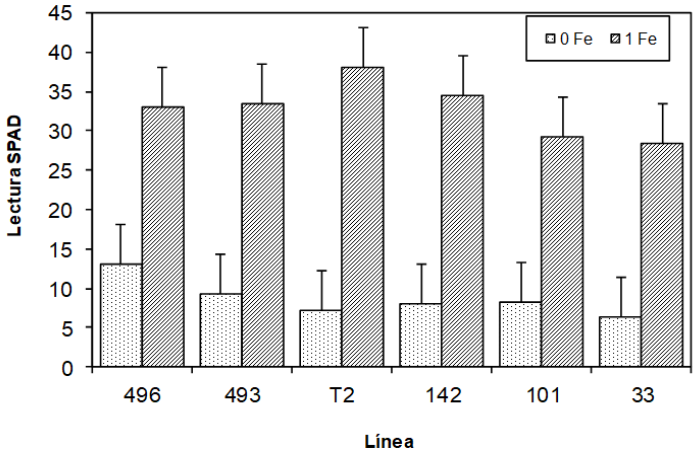

Figure 1. Leaf SPAD readings of bean lines and Fe in the solution.

The symptom of Fe deficiency in the foliage is a yellowing (chlorosis) of young leaves. Authors such as Dell'Orto *et al*. (2003) found in *Parietaria diffusa* plants grown in the absence of Fe that they showed chlorosis and a reduction in growth. Iron chlorosis causes a decrease in photosynthetic capacity, affecting pigments in different magnitudes (Abadía and Abadía, 1993). Chlorophyll reduction may be due to inhibition of the synthesis or degradation of pigments or their precursors (Bertamini and Nedunchezhian, 2005).

In beet plants susceptible to this physiopathy, the concentration of chlorophyll in the leaf decreases to a greater degree with respect to tolerant ones (Campbell and Nishio, 2000). Yellowing is due to an enrichment in carotenoids (xanthophylls). Xanthophyll synthesis works in Fe-deficient plants, but not in other yellowings, it also occurs in carotenoid-enriched materials; etiolated plants or senescent leaves (Abadía *et al*., 2000). In field conditions, the intensity of the symptoms does not always correlate with low concentration of Fe in the leaf and in some cases, this is even higher in chlorotic leaves than in green ones, due to an inactivation of Fe in the apoplast (Morales *et al*., 1998; Römheld, 2000; López-Millán *et al*., 2013).

Nutritional concentration of young leaves

Table 1 shows the results of the mineral composition of young leaves exposed to 0 and 1 mg L^{-1} of Fe and pH 6 and 8.5. With a Fe concentration of 1 mg L^{-1} in the nutrient solution, the concentration of Fe increased, but when it was 0 mg L^{-1} the leaf K, Ca, Mg, Mn, Zn, Cu and B increased. Fe deficiency stimulates the accumulation of K (Marschner, 1995; Maldonado-Torres *et al*., 2006), Mg (Belkhodja *et al*., 1998), Mn, Zn and Cu (Jolley *et al*., 2004) in young leaves.

Table 1. Leaf nutritional concentration of beans exposed to 0 and 1 mg L^{-1} Fe and pH 6 and 8.5.

	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
	(mg g^{-1})					(mg kg^{-1})				
Fe										
0	43.3 a	7.1 a	37.7 a	35.7 a	8 a	142 b	783.5 a	132 a	22.7 a	312.4 a
1	42.8 a	7.1 a	22.7 b	28.6 b	6.3 b	185.2 a	216.2 b	88.9 b	8.7 b	185 b
LSD	3.5	0.6	2.5	3.5	0.5	19.3	79.9	12.6	2.6	26.2
pH										
6	43.3 a	6.7 b	28.4 b	29.8 b	7.2 a	158.3 a	552.3 a	117.6 a	17.9 a	232.5 b
8.5	42.8 a	7.6 a	32 a	34.5 a	7.2 a	168.9 a	447.4 b	103.3 b	13.4 b	264.9 a
LSD	3.5	0.6	2.5	3.5	0.5	19.3	79.9	12.6	2.6	26.2

Values with the same letter, within the column, are statistically equal (Tukey, $\alpha = 0.05$).

The increase in K in the leaves is a consequence of an increased activity of the ATPase of the plasma membrane of the root involved in the extrusion of protons (Alcántara *et al*., 1991; Marschner, 1995; Blekhodja *et al*., 1998). Welkie *et al*. (1993) have associated the high concentration of K with the accumulation of organic acids in Fe-deficient plants. Thomas *et al*. (1998) found a higher concentration of K in chlorotic oak trees compared to non-chlorotic ones. For their part, in non-grass dicotyledons and monocotyledons with Fe deficiency that use strategy I, Alam *et al*. (2001); Marschner (1988) have determined a greater mobilization and absorption of Mn.

In the nutrient solution with pH 6, the leaf concentration of Mn, Zn and Cu increased, while at 8.5, P, K, Ca and B increased. The concentrations of N, Mg and Fe were not affected. At a low pH in the rhizosphere, roots absorb more cations than anions (Schaller, 1987; Schmidt *et al*., 2020). When cations are absorbed in excess with respect to anions, acidification of the rhizosphere occurs (Curtin and Wen, 2004). The pH of a nutrient solution affects dissociation, complexation and precipitation reactions, which significantly impacts the speciation and availability of essential elements, especially metal cations (Rijck and Schrevens, 1997).

In Table 2, the results show that where Fe was not added, bean line 33 had the lowest concentration of P, K and B, while line 493 presented a higher concentration of P and K. In the lines where Fe was added, there were no significant differences in leaf nutrient concentration. Although lines 496 and 33 had the same concentration of Fe, line 33 had a higher degree of chlorosis (Figure 1), indicating that it is inefficient in the use of Fe.

Table 2. Macro and micronutrients in young leaves in lines with 0 and 1 mg L^{-1} of Fe.

Fe	Line	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
		(mg g^{-1})					(mg kg^{-1})				
0	496	4.4 a	7.9 a	36.1 ab	33 abc	7.4 abc	160.2 abc	735.3 a	145.7 a	20.4 ab	272.9 bcd
0	493	5 a	7.8 a	41.8 a	27.9 bc	7.4 abc	117.9 bc	863.1 a	129.8 abcd	27 a	342.4 ab
0	T2	4.1 a	7.4 ab	41.9 a	44.4 a	8.3 ab	157.5 abc	896.5 a	132 abc	27.1 a	383.9 a
0	142	4.1 a	6.9 ab	40.3 ab	33.7 abc	9 a	125.5 bc	940.9 a	134.7 ab	23.8 a	329.9 ab
0	101	4 a	7.3 ab	35.1 abc	40.7 ab	8.8 a	181.8 abc	621.9 a	138.8 ab	16.8 abc	287.8 abc
0	33	4.4 a	5.3 b	31.3 bcd	34.4 abc	7 abc	109.1 c	643.2 a	111 abcde	21.1 ab	257.6 bcd
1	496	4.3 a	7.2 ab	21.7 de	26.4 bc	5.9 c	179.5 abc	174.1 b	81.6 cde	8.6 c	173.9 d
1	493	4.5 a	7 ab	23.3 de	24.1 c	5.7 c	169.7 abc	190.6 b	75.6 e	7.6 c	177.3 d
1	T2	3.8 a	6.2 ab	19.3 e	33.9 abc	6.6 bc	196.3 ab	240 b	87.9 bcde	8 c	202.7 cd
1	142	4.6 a	8.1 a	22.9 de	32.7 abc	7.2 abc	219.8 a	217.2 b	116.4 abcde	8.8 c	180.2 cd
1	101	4.5 a	7.3 ab	25.3 cde	28.6 bc	6.4 bc	161.5 abc	264.1 b	93.6 abcde	10.7 bc	183.2 cd
1	33	4 a	6.9 ab	23.4 de	25.7 c	6.3 bc	184.5 abc	211.4 b	78.5 de	8.2 c	192.8 cd
	LSD	1.5	2.4	10.5	14.6	2.2	80	331.9	52.4	10.8	108.8

Values with the same letter, within the column, are statistically equal (Tukey, $\alpha = 0.05$).

The leaves with chlorosis show physiological alterations, such as a decrease in the concentration of chlorophylls, radiation capture and photosynthetic capacity. Light absorbed and not used in photosynthesis can photo-inhibit processes and photo-oxidize compounds (Abadía *et al*., 2000; Rout and Sahoo, 2015). In chlorotic leaves, Fe accumulates in unavailable forms (Marschner, 1991).

Cells of chlorotic leaves have a limited capacity for the absorption of Fe, and this could be accumulated in the apoplast, which can be amplified under field conditions. Thus, this accumulation in chlorotic leaves would be the consequence of the enrichment of Fe in leaves when the plant no longer has the capacity to use it (Bohórquez *et al*., 2001).

Some chlorotic species green when dilute acid is applied to the petiole and when the leaves are treated with compounds that promote the functioning of plasma membrane ATPases (Kosegarten, 1999). In Fe-deficient plants, high pH in the cytoplasm has been reported and this is associated with immobilization (Abadía, 1992).

The concentration of Mn in stem and root follows an inverse relationship with Fe, the mobilization and absorption of Mn increases during Fe deficiency in plants that use mechanism I (Alam *et al*., 2001). In Figure 2, the plants with nutrient solution without Fe show a generally higher Nutrient Imbalance Index (NII), but where Fe was added, lines 496 and 33 had the lowest indices, while in T_2 it was higher.

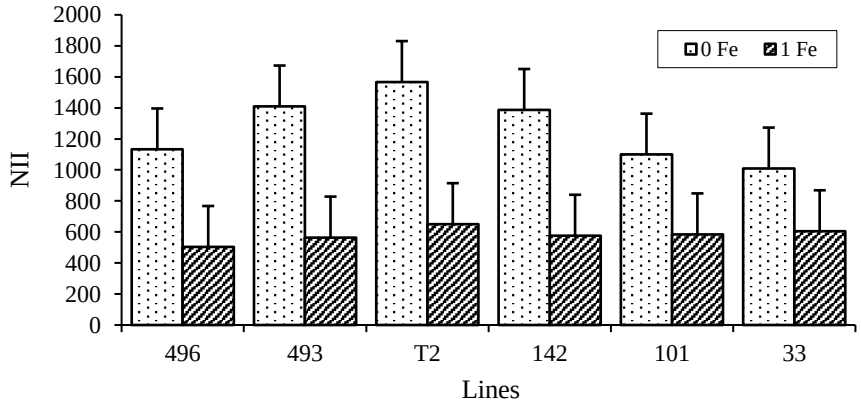

Figure 2. Interaction of bean lines and Fe in the nutritional imbalance index (NII).

This was contrary to SPAD readings, where T_2 values were higher. Although Fe was not applied, line T_2 had the same degree of chlorosis (Figure 1) as line 33 but had a higher NII. Conversely, it occurred when Fe was applied, since T_2 had higher NII and SPAD readings, while in line 33, NII and SPAD readings were lower, so T_2 adapted better to Fe deficiency than line 33.

Regarding nutritional ratios, it was found that where Fe was not added, the ratios N/P, B/P and Ca/P were higher in line 33 (Table 3), which indicated that in this there was a higher amount of P in the young leaves, while line 496 was lower. High concentrations of P in the medium or plant increase iron chlorosis (Clark, 1991). Keshirad *et al*. (1978) suggest that high doses of P interfere with the absorption and translocation of Fe, inactivating and precipitating it in the apoplast of chlorotic plants, preventing its translocation to mesophyll cells.

Table 3. Nutritional ratios obtained in bean lines.

Fe (mg L^{-1})	Line	N/P	P/Mg	Cu/P	B/P	Ca/P	Mn/Ca	B/Mg	Mn/Fe
0	496	5.8b	1.15 abc	1.19 c	35.2 bcd	4.3 bcde	22 abc	40.4 ab	5.1 bcd
0	493	7 ab	1.1 abc	1.48 c	43.7 ab	3.7 cde	31.9 a	47.6 a	7.7 ab
0	T2	6 b	0.89 bcd	1.11 c	54.5 a	6.1 ab	20.7 bc	46.6 a	5.9 abc
0	142	6.2 b	0.78 d	1.34 c	48.3 ab	4.9 bcde	29.8 ab	37.9 ab	8.9 a
0	101	5.7 b	0.84 cd	1.11 c	39 abc	5.6 abc	16.9 cde	33.2 bc	4 cde
0	33	8.9 a	0.77 d	1.24 c	52.1 a	6.9 a	18.7 cd	37.6 ab	5.9 abc

Fe (mg L^{-1})	Line	N/P	P/Mg	Cu/P	B/P	Ca/P	Mn/Ca	B/Mg	Mn/Fe
1	496	6.2 b	1.22 a	4.23 a	24.1 cd	3.7 cde	6.6 f	29.3 bc	1 e
1	493	6.4 ab	1.26 a	2.41 bc	25.5 cd	3.4 e	7.9 ef	31.9 bc	1.1 e
1	T2	6.3 b	0.95 abcd	3.5 ab	33.4 bcd	5.4 abcd	7.2 ef	31.2 bc	1.2 e
1	142	5.8 b	1.14 abc	3.88 ab	22.2 d	4 cde	6.6 f	25.1 c	1 e
1	101	6.3 b	1.17 ab	3.97 ab	25 cd	3.9 cde	9.7 def	28.8 bc	1.7 de
1	33	6 b	1.15 abc	2.53 bc	27.6 cd	3.6 de	8.1 ef	31.6 bc	1.1 e
	LSD	2.6	0.31	1.7	15.7	1.9	10.1	12.1	3.6

Values with the same letter, within the column, are statistically equal (Tukey, α= 0.05).

In chlorotic plants, the ratios P/Fe, K/Ca, K/Fe, Mn/Fe, Cu/Fe and Zn/Fe were higher (Table 4). This indicates a lower amount of Fe compared to other cations (K, Mn, Cu and Zn) and a higher amount of K, P and Mn, which coincides at least for the P/Fe ratio, since Köseoglu (1995) and Maldonado-Torres *et al*. (2006) found that this ratio is higher in chlorotic leaves than in green leaves. It has been found that the K/Ca ratio is high in pear tree leaves with iron chlorosis (Abadía *et al*., 1989), peach (Belkhodja *et al*., 1998) and Mexican lime (Maldonado-Torres *et al*., 2006).

Table 4. Nutritional ratios with Fe treatments on bean lines.

Fe	P/Fe	K/Ca	K/Fe	Mn/Fe	Cu/Fe	Zn/Fe
0	0.055 a	1.2 a	0.31 a	6.23 a	0.18 a	1.01 a
1	0.04 b	0.9 b	0.13 b	1.19 b	0.05 b	0.46 b
LSD	0.007	0.2	0.04	0.87	0.02	0.11

Values with the same letter, within the column, are statistically equal (Tukey, α= 0.05).

It has often been verified that the chlorotic organs have high concentrations of K (Loué, 1988), this effect is attributed to the fact that excess K in the leaves can displace Fe from the metabolic surfaces of enzymes (El-Kassas, 1984), but K deficiency is considered a susceptibility to iron chlorosis. K favors the absorption of Fe as a result of acidification of the rhizosphere soil and an increase in the plant in the citric acid that transports Fe within the plant (Marschner, 1995).

Mineral composition in the root

The addition of 1 mg L^{-1} of Fe to the nutrient solution influenced the increase in the concentration of N, P, K and Fe (Table 5). In the roots of plants that did not receive Fe, Mn, Zn and Cu increased, while the concentrations of Ca, Mg and B were not influenced. Alhendawi *et al*. (1997) examined that the concentration of K^+ and NO_3^- in the roots is decreased by HCO_3^-, due to an increase in the flow of these ions into the medium. High concentrations of CO_3^{2-} and HCO_3^- (alkaline pH) induce low availability of nutrients such as Fe and sometimes P (Dell'Orto *et al*., 2003; Parrilla and Schmidt, 2019).

Table 5. Average nutritional concentration in root tissue of bean plants (mg L^{-1}).

	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
Fe										
0	35.5 b	15.1 b	34 b	14.1 a	13.2 a	1125.5 b	1887.7 a	478.9 a	248 a	185.1 a
1	41.7 a	22.1 a	37 a	12.5 a	13.4 a	1427.8 a	983.1 b	189.8 b	42.3 b	166.8 a
LSD	1.5	2.4	2.2	1.6	1.3	284.1	213.8	67.4	25.6	32.1
pH										
6	39.1 a	20.6 a	35.4 a	12 b	12.7 a	1245.7 a	1340.9 a	320.8 a	146.3 a	173 a
8.5	38 a	16.5 b	35.6 a	14.7 a	13.8 a	1307.6 a	1529.9 a	347.8 a	144 a	178.9 a
LSD	1.5	2.4	2.2	1.6	1.3	284.1	213.8	67.4	25.6	32.1

Values with the same letter, within the column, are statistically equal (Tukey, α= 0.05).

Alhendawi *et al*. (1997) mention that increasing the concentration of HCO_3^- decreases the content and concentration of Fe in the roots and a similar percentage in the stem. This indicates that the reduction of Fe induced by HCO_3^- in the stem is not a specific effect of translocation of Fe from the root to the stem, but in the absorption of Fe by the root. Alam *et al*. (2001) found that the concentration of Mn is higher than in the root in plants with and without Fe. The pH of six in the solution increased the concentration of P and at a pH 8.5, that of Ca increased. The concentration of other nutrients was affected by the pH.

The line T_2 reached a higher concentration of N and B in the roots of plants without application of Fe (Table 6). Regarding K, lines 496, 493 and 33 were the ones that obtained the highest concentration and T_2 the lowest. The concentration of Fe and Mg was higher in line 496. Line 142 had a higher concentration of Mn and Zn, but was the lowest in Fe, which agrees with different authors who mention that the concentrations of Mn and Fe follow an inverse relationship (Roomizadeh and Karimian, 1996; Foy *et al*., 1998; Ghesemi-Fasaei *et al*., 2003). Line 493 had the highest concentration of Fe in the root when they received Fe. However, the degree of chlorosis was the same as that of the lines with smaller magnitude (Figure 1).

Table 6. Mineral composition of bean root with 0 and 1 mg L^{-1} of Fe in the nutrient solution.

Fe	Line	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
		(mg g^{-1})					(mg kg^{-1})				
0	496	34.4 f	15.7 def	37.1 ab	12.6 ab	15.8 a	1886.8 ab	1989.9 ab	406.6 b	241.1 a	148.6 b
0	493	34.9 ef	17.9 cdef	36.2 ab	13 ab	14.5 ab	1418.7 bc	2170.1 a	509.6 ab	253.7 a	164.3 ab
0	T2	38.5 cde	12.8 f	29.6 c	14.1 a	14.5 ab	685.9 de	1868.2 ab	517.3 ab	238.7 a	198.9 a
0	142	33.6 f	15.3 def	32.9 abc	14.1 a	10.8 cd	461.4 e	2162.5 a	619.8 a	269.8 a	171.5 ab
0	101	35 ef	13.7 ef	32 bc	15.3 a	10 d	704.8 de	1469.9 bc	411.8 b	235.7 a	169.3 ab
0	33	36.3 def	15 def	36.5 ab	15.5 a	13.6 abc	1595.4 bc	1665.5 ab	408 b	249 a	169.7 ab
1	496	41.1 abc	24.5 ab	37.5 ab	12.1 ab	14.4 ab	1548.6 bc	1013.6 cd	196.9 c	44.9 b	187.1 ab
1	493	41.4 abc	26.3 a	36.9 ab	13.4 ab	13.7 abc	2295.9 a	1040.2 cd	170.4 c	38.4 b	148.1 b
1	T2	41.4 abc	20.6 abcd	38.1 a	13.4 ab	14.4 ab	1017.9 cde	967.1 cd	209.1 c	36.1 b	163 ab

Fe	Line	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
		(mg g^{-1})					(mg kg^{-1})				
1	142	44.1 a	22.1 abc	36 ab	13 ab	12.4 bcd	945.8 cde	1017.5 cd	182.8 c	36.6 b	181.4 ab
1	101	42.4 ab	20 bcd	37.5 a	10.1 b	13.4 abc	1490.1 bc	868.5 d	173.5 c	33.3 b	170.3 ab
1	33	39.6 bcd	19 bcde	35.9 ab	13.3 ab	11.8 bcd	1268.3 bcd	992.1 cd	205.9 c	64.6 b	150.9 b
LSD		3.6	5.8	5.4	4	3.1	695.9	523.7	165	62.8	41.7

Values with the same letter, within the column, are statistically equal (Tukey, α= 0.05).

The transfer coefficient of all nutrients increased in chlorotic leaves (Table 7), so, Fe deficiency increases their translocation to young leaves. The root and stem interact by transferring nutrients between vessels of xylem that nourish the leaves and those to the stem (Jeschke and Hartung, 2000).

Table 7. Coefficient of nutrient transfer between young leaves and root of beans.

Fe	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
0	1.24 a	0.53 a	1.18 a	2.77 a	0.69 a	0.26 a	0.47 a	0.52 a	0.25 a	1.94 a
1	1.03 b	0.36 b	0.64 b	2.4 b	0.5 b	0.16 b	0.25 b	0.34 b	0.1 b	1.3 b
LSD	0.11	0.07	0.11	0.34	0.09	0.09	0.07	0.07	0.03	0.27

Values with the same letter, within the column, are statistically equal (Tukey, α= 0.05).

The content and concentration of Fe in barley, sorghum and corn in all parts of the plant are significantly reduced by elevated levels of bicarbonate (Alhendawi *et al*., 1997). Alhendawi *et al*. (1997) observed that the supply of bicarbonates results in an accumulation of organic acids in roots and a decrease in the absorption and translocation of Fe. Bicarbonate has been found to avoid the translocation of Fe to young organs and the transport of Fe from the apoplast within the cytoplasm to the apex level (Mengel, 1994), although it is not a general thesis (Römheld, 2000). Toulon *et al*. (1992) and Susin *et al*. (1996) have shown that the reduction of Fe^{3+} in the apoplast of intact roots occurs at low pHs.

Root volume

In plants without Fe, there was no statistically significant difference (Figure 3), but where Fe was present, lines 496 and 33 had greater root volume, while in line 101 it was lower. Fe deficiency produces physiological responses in the root (Abadía *et al*., 2000; Tripathi *et al*., 2018), which include transformation of epidermal roots into transfer cells (Schmidt and Bartels, 1996), acidification of the rhizosphere (Alcántar *et al*., 1991) and increased iron reduction capacity of roots (Robinson *et al*., 1999).

The development of morphological and physiological changes in the root is another response found in Fe-deficient dicotyledons. The proliferation of root hairs is a modification induced by Fe deficiency, in plants that carry out strategy I (Landsberg, 1996). In *Casuarina glauca*, Fe deficiency is one of the main factors, in addition to P, in the induction of cluster root (proteoids) formation, even reversing cluster root formation due to P deficiency due to the addition of Fe to the nutrient medium. In addition, the formation of this type of roots appears to be an efficient morphological response to Fe stress (Arahou and Diem, 1997; Connorton *et al*., 2017).

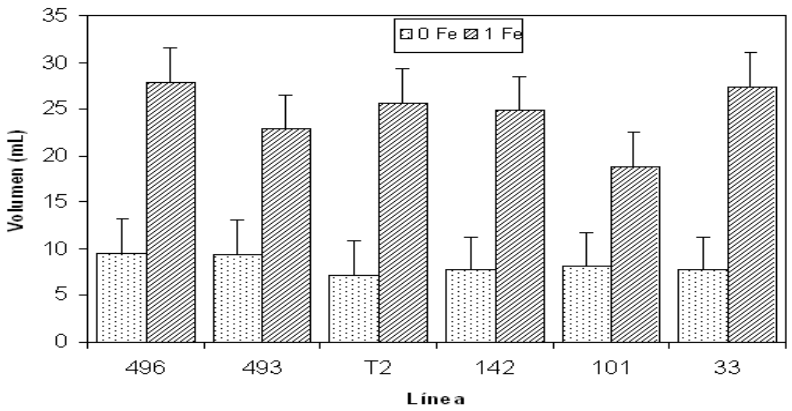

Figure 3. Effect of bean line and iron concentration on root volume.

Campbell and Nishio (2000) found that plants under Fe deficiency decrease the growth of the root tip, retardation of root tissue growth and production of lateral roots, in addition to the formation of numerous root hairs, which correlates with a significant decrease in chlorophyll. These roots produce and excrete phosphatases and organic acids that acidify the soil and chelate minerals, mobilizing P and other micronutrients. The detriment of the acquisition of Fe may be a consequence of the inhibition of root growth (Alhendawi *et al*., 1997).

Bicarbonates absorbed through the roots become used in the synthesis of organic acids, being fixed *in situ* by phosphoenolpyruvate carboxylase (PEPase) to form oxaloacetate, which is reduced to produce malate (Cramer *et al*., 1993). In rice, inhibition of root growth due to the high bicarbonate concentration is linked to high concentrations of organic acids in the roots (Wairich *et al*., 2019).

Dry matter

There were no significant differences in dry matter in the plants that grew without Fe supply (Figure 4). When Fe was added, lines 496, 33 and T_2 produced more dry matter and the lowest value corresponded to line 101. This agrees with the root volume, as they have the same tendency, thus a good root system causes a higher production of biomass. However, only in T_2 were SPDAD readings higher. (Belkhodja *et al*., 1998; Connorton *et al*., 2017) mention that the decrease in chlorophyll concentration is not always due to Fe deficiency, but to a dilution effect; and that it can be the result of two processes; a dilution of chlorophyll caused by growth and subsequent inability to produce or stabilize new molecules in thylakoid membranes.

In barley, sorghum and corn, it was reported that increases in the concentration of bicarbonate ions in the nutrient medium promote iron chlorosis, significantly decrease the dry biomass of root and stem (Alhendawi *et al*., 1997; Jia *et al*., 2018; Li *et al*., 2021). (Bertamini and Nedunchzhian, 2005; Riaz *et al*., 2021) found a 37% reduction of dry biomass in Fe-deficient grape leaves.

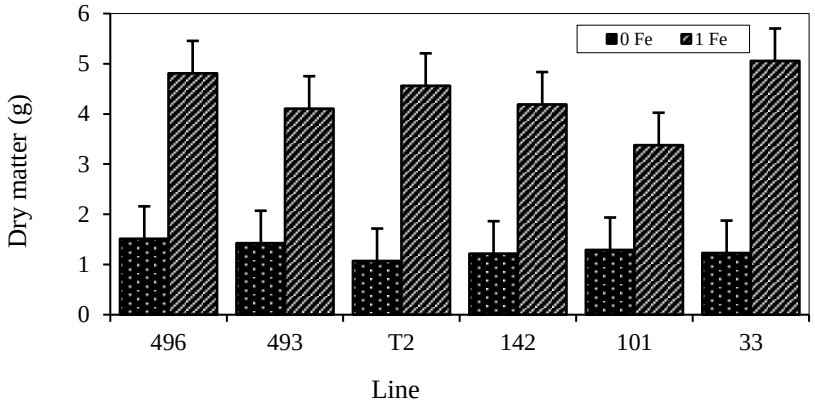

Figure 4. Dry biomass production of bean lines exposed to deficiency and supply of Fe.

Conclusions

Iron chlorosis increases the NII, transfer coefficient, the ratios P/Fe and K/Ca, and the concentration of K, Ca, Mg, Mn, Zn, Cu, and B in young bean leaves. In the absence of Fe, line 496 presents lower chlorosis, the P/Mg ratio and the concentration of P and K increase. When Fe is present in the nutrient solution, while lines 496 and 33 had low nutritional indices and higher dry biomass production.

The line T_2 was susceptible to Fe deficiency, but with doses of 1 mg L^{-1} in the solution it had greater production of dry biomass, root volume and did not show iron chlorosis. Line 33 was classified as susceptible and the ratios N/P, B/P, Ca/P increased and the concentration of P, K and B decreased. The addition of 1 mg L^{-1} of Fe increased the concentration of N, P, K and Fe and without supply of Fe, the concentration of Mn, Zn and Cu in root increased.

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