The effect of high iron doses (Fe^{2+}) on the growth of broccoli plants (*Brassica oleracea* var. *Italica*)

Efecto de dosis altas de hierro (Fe^{2+}) sobre el crecimiento de plantas de brócoli (*Brassica oleracea* var. *Italica*)

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ABSTRACT

Tests were carried out under greenhouse conditions in Tunja (Colombia) in order to evaluate the effect of Fe^{2+} toxicity on the growth of broccoli plants. 'Legacy' hybrid *Brassica oleracea* var. *Italica* plantlets were grown in glass containers with a nutritive solution. Iron sulfate was added to the substrate in order to produce excess iron at concentrations of 100 and 200 mg L^{-1}; a control without iron sulfate applications was used. The following evaluations were made: leaf area, total dry weight of the plants, distribution of dry mass (DM) in the different organs, absolute growth rate, relative growth rate, net assimilation rate and the root:shoot ratio. The total DM decreased drastically in the plants subjected to excess Fe^{2+}, the growth indices progressively decreased with increases in the Fe^{2+} concentrations in the substrate and the distribution of DM in the organs varied as a function of the needs of the plants, with 15.85 and 11.10% less DM in the roots of the plants subjected to Fe^{2+} than in the control plants, at 100 and 250 mg L^{-1}, respectively.

Key words: plant nutrition, dry mass partitioning, iron sulfate, toxicity to plants.

RESUMEN

Con el objetivo de evaluar el efecto de la toxicidad por Fe^{2+} sobre el crecimiento de plantas de brócoli, se llevó a cabo un ensayo bajo condiciones de invernadero en Tunja (Colombia). Se utilizaron plántulas de *Brassica oleracea* var. *Italica* híbrido 'Legacy', sembradas en contenedores de vidrio con solución nutritiva. Se adicionó sulfato de hierro al sustrato para inducir el exceso del metal, en concentraciones de 100 y 250 mg L^{-1} y un control sin aplicación de sulfato de hierro. Se evaluó el área foliar, el peso seco total de la planta y la distribución de la materia seca (MS) en los diferentes órganos, se calculó la tasa de crecimiento absoluto, tasa de crecimiento relativo, tasa de asimilación neta y la relación raíz:vástago. El peso seco total disminuyó drásticamente en las plantas sometidas a exceso de Fe^{2+}, los índices de crecimiento disminuyeron progresivamente a medida que aumentó la concentración de Fe^{2+} en el sustrato y la distribución de la MS en los órganos varió en función de las necesidades de la planta, siendo 15,85 y 11,10% menor la proporción de MS en las raíces de las plantas sometidas a toxicidad por Fe^{2+} que en las plantas control, para 100 y 250 mg L^{-1}, respectivamente.

Palabras clave: nutrición de los cultivos, distribución de la materia seca, sulfato de hierro, toxicidad de las plantas.

Introduction

Toxicity due to excess iron is a frequent problem in acid soils (Nenova, 2006) and can be caused by microbial action in flooded soils, which results in the reduction of insoluble Fe^{3+} in favor of soluble Fe^{2+}, which can be taken up by the roots of plants in excessive quantities (Becker and Asch, 2005). In addition, when the content of this metal is high in the soil, the symtomatology seen in affected plants corresponds to deficiencies of other elements considered as antagonistic to iron, such as in the case of Mn (Hanke, 2008), P and ZN, in addition to H_2S toxicity conditions (Kirk, 2004).

Currently, the relationship between iron toxicity severity, expressed symptoms and crop yield has not been clearly established (Asch *et al.*, 2005). However, the principal symptom associated with Fe^{2+} is called browning or yellowing of the leaves, fundamentally characterized in *Oryza sativa* plants, which causes a reduction in growth, principally in height and tillering, and an increase in sterility of the panicle (Audebert, 2006a). In addition, the physiological behavior of the plants under Fe^{2+} toxicity conditions has been documented for various vegetative species under laboratory and natural conditions, fundamentally based on parameters such as the accumulation of dry and fresh mass, root and shoot lengths, number of leaves, and leaf

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area (Nenova, 2006). Similarly, excess iron toxicity in the soil has been related to the behavior of photosynthetic apparatus in broccoli plants, principally based on measurements of the fluorescence of chlorophyll *a* (Peña-Olmos and Casierra-Posada, 2013).

Taking into account the fact that, in Colombia, the majority of soils have acidity problems (Casierra-Posada *et al.*, 2008) and that excess iron toxicity is a frequent limitation associated with low pH values in the soil (Nenova, 2006). The present study aimed to evaluate the growth of *Brassica oleracea* var. *Italica* plants subjected to excess Fe^{2+} stress.

Materials and methods

The evaluation was developed in a glass greenhouse of the Facultad de Ciencias Agropecuarias of the Universidad Pedagogica y Tecnologica de Colombia - UPTC (Tunja, Colombia), with an average temperature inside the greenhouse of 15.8°C, 72.0% relative humidity and an average photosynthetically active radiation (*PAR*) of 1,380 µmol m^{-2} s^{-1}. One-month-old 'Legacy' hybrid broccoli plantlets (*Brassica oleracea* var. *Italica*) were used as the initial plant material, sown in glass containers with a nutritive solution of the following composition in mg L^{-1}: 40.3 nitric nitrogen; 4.0 ammonical nitrogen; 20.4 phosphorus; 50.6 potassium; 28.8 calcium; 11.4 magnesium; 1.0 sulfur; 1.12 iron; 0.012 manganese; 0.012 copper; 0.0264 zinc; 0.106 boron; 0.0012 molybdenum and 0.00036 cobalt.

Approximately 20 d after transplant, iron sulfate was added to the plants at concentrations of 100 and 200 mg L^{-1} in order to induce excess iron; a control without an iron sulfate application was also used. The pH of the solutions was 5.5, 5.3 and 6.2, respectively. The iron additions were done progressively in order to avoid damaging the plants. 44 d after transplant, the leaf area was determined using an LI-3000-A® integrated meter (LI-COR, Lincoln, NE), the DW of the roots, the stems, the leaves and total DW were also determined through oven drying at 105°C until constant weight. In addition, the distribution of the dry material in the different organs was determined as a percentage of the DW assigned to each organ in respect to the total DW of the plant. Based on the DW, the root:shoot ratio was calculated as a coefficient between the root DW and the aerial parts DW. At the begining of the experiment, the initial leaf area and DW were recorded for 20 plants in order to have the initial values needed for the calculation of the growth indices (absolute growth rate (AGR), relative growth rate (RGR) and net assimilation rate [NAR]).

The greenhouse was prepared using tubes and hoses for the connection of an aeration system for the glass containers in order to oxygenate the nutritive solution of the plants. The experimental units were arranged with a completely random design with three treatments and 15 repetitions per treatment. An analysis of variance (ANAVA) was carried out and the treatments were compared using a Tukey mean comparison test ($P \leq 0.05$). The statistical analysis was done with version 19.0.0 of IBM-SPSS statistics (IBM Corporation, New York, NY).

Results and discussion

The total DW of the plants was markedly lower in the plants subjected to iron toxicity; the concentrations of 100 and 250 mg L^{-1} of the metal induced a reduction in the total DM of the plants of 73.63 and 84.81%, respectively, in regard to the control plants ($P \leq 0.05$) (Fig. 1).

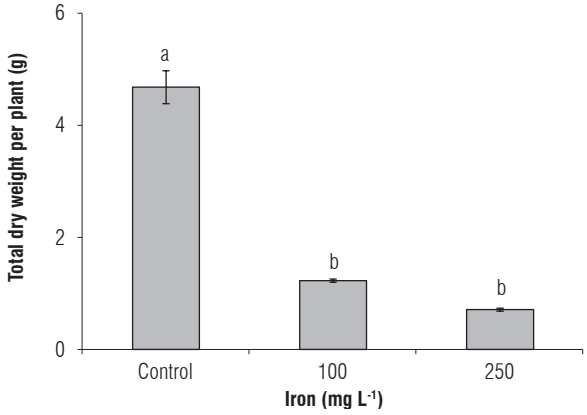

FIGURE 1. Total dry weight of the broccoli plants subjected to iron toxicity. Means with different letters indicate significant differences according to the Tukey test ($P \leq 0.05$) (n = 15). Error bars indicate standard error.

Mehraban *et al.* (2008) found that elevated concentrations of Fe^{2+} in the substrate strongly decreased radicle development, supported on the DW, especially when the Fe^{2+} doses surpassed 50 mg L^{-1} because high concentrations of the metal increased the peroxidation of lipids, particularly in the radicle zone, which was accompanied by stunted growth of the same. This radicle behavior affected the complete development of the plants because reductions in radicle volume and losses of radicle hairs can induce a drastic decrease in the relative content of water in plants (Dorlodot *et al.,* 2005).

In rice, the DW of roots, veins and foliar lamina did not demonstrate significant differences when the plants were subjected to high iron concentrations in the substrate

(Majerus *et al*., 2007). However, the progressive decrease in the DM in the roots, stems, and leaves in the present study is evidence of impeded growth, produced by a high flow of Fe^{2+} ions towards the interior of the plant. Becker and Asch (2005) indicated that a strategy used by rice plants to offset excess Fe^{2+} is the accumulation of the metal in the stems and leaves; highly elevated concentrations of this metal cause early defoliation in these plants in an attempt to attenuate the toxic effects in the plant with a consequent decrease, and in some cases complete cessation of growth in different organs of the plant, as occurred in the present study.

Decreases in the total DW of pea plants were reported by Nenova (2006) at 41 d after sowing and with a Fe^{2+} concentration of 40 mg L^{-1} in the substrate. This was due to the fact that the iron toxicity induced the formation of reactive oxygen species, which could be the cause of the growth inhibition. However, Batty and Younger (2003) confirmed that growth inhibition may occur before Fe^{2+} concentrations in the plant tissues are toxic, which suggests that a strong flow of Fe^{2+} towards the plant tissues creates a barrier that impedes the entrance of other nutrients that are essential for the growth and development of the plants. For example, in the cultivation of rice, high applications of Fe^{2+} in the substrate increase the concentrations of the metal in the roots, veins and foliar lamina, which reduce the concentrations of K, Ca, Mg, Mn and P in the shoots of the plants, which affects the growth and development of the crop (Majerus *et al*., 2007).

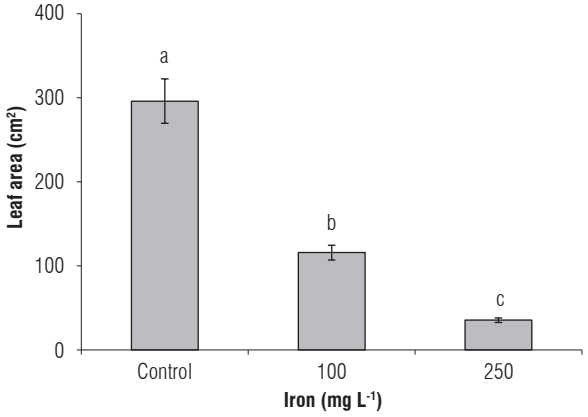

FIGURE 2. Leaf area of the broccoli plants subjected to iron toxicity. Means with different letters indicate significant differences according to the Tukey test ($P \leq 0.05$) (n = 15). Error bars indicate standard error.

The control plants presented the highest foliar area value, followed by the plants subjected to the 100 and 250 mg L^{-1} Fe^{2+} concentrations in the nutritive solution, in which the values were reduced by 60.87 and 87.98%, respectively ($P \leq 0.05$) (Fig. 2).

By definition, leaves are reserve organs and Fe^{2+} is an immobile element, and so is constantly accumulated within the vacuoles of foliar tissue cells and cannot be redistributed during senescence (Audebert, 2006b). The strong decrease in the leaf area of the plants subjected to excess iron in the preset study suggests that the constant flow of the metal through the conductor tissues of the plants was sufficiently strong to cause premature loss of the leaves in the evaluated plants. This may have been due to the low levels of soluble protein, total soluble sugars, chlorophyll *a, b* and total chlorophyll, as induced by the Fe^{2+} (Mehraban *et al*., 2008), which decreased foliar growth and expansion and the useful leaf life, and, consequently, caused a reduction in crop yield (Audebert, 2006b). Similarly, excess iron toxicity induced an increase in the lipids peroxidation of leaves in *Eugenia uniflora* (De Oliveira-Jucoski *et al*., 2013), which affected the structure and process of cellular division, drastically decreasing foliar expansion.

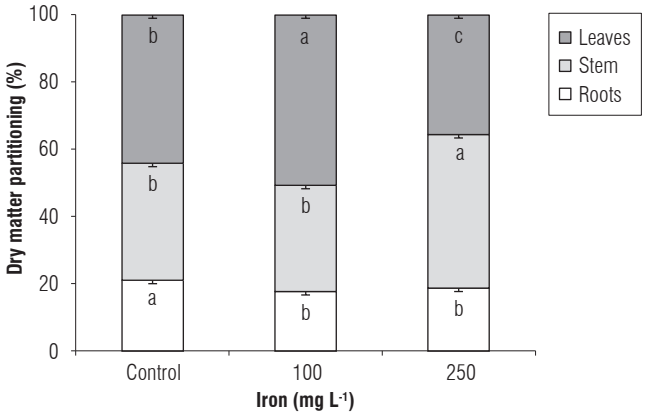

FIGURE 3. Dry matter partitioning in broccoli plants subjected to iron toxicity. Means with different letters indicate significant differences according to Tukey test ($P \leq 0.05$) (n = 15). Error bars indicate standard error.

The assignment of DM to the different organs presented significant differences between the evaluated treatments ($P \leq 0.05$) (Fig. 3). The distribution of photoassimilates towards the leaves increased with the 100 mg L^{-1} Fe^{2+} application in the substrate, while the 250 mg L^{-1} addition produced a higher flow of photosynthates towards the stem and lower development in the leaves and roots, respectively.

The reduction of the DM percentage destined for the roots in the plants subjected to iron toxicity in the present study corresponds with the high accumulation of Fe^{2+} in the plants, which reduced plant growth (Mehraban *et al*., 2008); however, the chlorophyll contents also decreased as a consequence of oxidative stress (Gajewska and Skłodowska, 2007), in this case, due to the increase of

the Fe^{2+} concentration in the plant tissues (Mehraban *et al*., 2008), which would limit the photosynthetic activity of the same and also cause a smaller proportion of photoassimilates produced in the leaves and translocated towards the roots for growth and development. Peña-Olmos and Casierra-Posada (2013), who worked with the same plants as the present study, found that the electron transport rate (ETR) in the leaves decreased drastically with an increase in the iron concentration of the substrate, which directly affected the photosynthetic activity of the plants.

The increase in the percentage of DM in the leaves of the plants subjected to 100 mg L^{-1} Fe^{2+} could be the result of a compensatory behavior of the plants, due to the drastic decrease of the radicle development and the consequent decrease in the uptake of essential nutrients as a result of the high flow of Fe^{2+} ions towards the interior of the plants requiring a higher efficiency of the same in the capture of light in order to supplement the nutritional requirements throughout the structure, offsetting the low radicle development and producing an osmotic readjustment in the plant. Peña-Olmos and Casierra-Posada (2013), found in broccoli that the maximum quantum efficiency of photosystem II (F_v/F_m) decreased notably with an increase in the Fe^{2+} concentration of the substrate, concluding that the stress levels of the plants were elevated, possibly due to the accumulation of Fe^{2+} ions in plant tissues. Many studies have reported that abiotic stress induces an increase in accumulated sugars in the aerial parts of plants and exercises a strong influence on the partition of assimilates in different plant species (Majerus *et al*., 2007).

For example, Majerus *et al*. (2007) found that the concentration of soluble sugars decreased in the roots with the application of Fe^{2+}, reaching 80% less in plants stressed with 500 mg L^{-1} Fe^{2+} in comparison with the control plants. However, the same authors indicated that the concentrations of soluble sugars increased with Fe^{2+} applications both in the veins and in the foliar lamina. This increase in the concentration of soluble sugars in leaves, added with the decrease of same in the roots, suggests that sucrose load and its subsequent translocation from the aerial part towards the leaves are inhibited under conditions of excess Fe^{2+} stress, especially in susceptible crops. An increase in concentrations of sucrose, glucose and fructose, as a result of a modification in the activities of the sucrose phosphate synthase, sucrose synthase and invertase, was found in *Lupinus albus* leaves subjected to a water deficit (Pinheiro *et al*., 2001), which, possibly deals with the protection of cellular structures and/or with the osmotic regulation of plants under conditions of severe stress. This did not occur in the plants under 250 mg L^{-1} Fe^{2+}, possibly due to fact that the concentration of the metal was so high that it almost completely stopped vegetative growth.

The highest accumulation of DM which was found in the stems of the plants treated with 250 mg L^{-1} Fe^{2+} may have possibly been due to the plants having two options in the presence of stress induced by excess iron. The first is to tolerate the elevated concentrations of iron in the foliar tissues and the second is to create a barrier to impede the entrance of excess iron into the vegetative tissues. A tolerance to excess iron in broccoli has yet to have been reported; therefore, it is possible to think that the second option is more likely for the counteraction of iron toxicity. This, due to the fact that plants create a barrier of oxidation at the rhizosphere level in order to decrease the entrance of Fe^{2+} ions towards the vegetative tissues, was stabilized by the canalization of molecular oxygen through the stem and towards the roots, using gas conducting tissue or aerenchyma (Becker and Asch, 2005); for which, plants under conditions of excessive Fe^{2+} in the substrate must invest a higher percentage of photoassimilates in the formation of gas conducting tissue in the stem.

Table 1 presents the absolute growth rate (AGR), the relative growth rate (RGR) and the net assimilation rate (NAR) of the evaluated broccoli plants. The AGR presented significant differences between the treatments ($P \leq 0.05$) with the 100 and 250 mg L^{-1} Fe^{2+} applications producing decreases in this variable of 74.77 and 85.98%, respectively. Similarly, the 100 and 250 mg L^{-1} Fe^{2+} applications caused a 28.89 and 42.22% decrease in the RGR, respectively ($P \leq 0.05$). The Fe^{2+} toxicity induced a decrease in the NAR value with the addition of 100 mg L^{-1} of 49.09 and 40.00% with the application of 250 mg L^{-1}.

Nenova (2006) found in pea plants that, 34 d after the induction of toxicity with 40 mg L^{-1} Fe^{2+}, the AGR value decreased 69.12% in comparison with the plants subjected to 2 mg L^{-1} Fe^{2+}. This was possibly related to a decreased in the electron transport rate, affecting the Calvin cycle and causing a decrease in the carboxylation:oxigenation ratio of Rubisco, which would favor photorespiration (Kampfenkel *et al*., 1995). Peña-Olmos and Casierra-Posada (2013) found that, in broccoli, the electron transport rate (ETR) decreased notably with an increase in Fe^{2+} concentration in the substrate. Furthermore, these authors determined the photochemical extinction coefficient (qP) and concluded that the value of this variable is inversely proportional to the Fe^{2+} content in the substrate, which directly affects the photosynthetic capacity of the plant as well as its growth.

TABLE 1. Absolute growth rate (AGR), relative growth rate (RGR) and net assimilation rate (NAR) of broccoli plants subjected to iron toxicity.

Iron ($mg\ L^{-1}$)	Absolute growth rate ($g\ d^{-1}$)	Relative growth rate ($g\ g\ d^{-1}$)	Net assimilation rate ($g\ cm^{-2}\ d^{-1}$)
Control	$0.107 \pm 6.9 \cdot 10^{-3}$ a	$0.045 \pm 7.0 \cdot 10^{-4}$ a	$5.5 \cdot 10^{-4} \pm 2.0 \cdot 10^{-5}$ a
100	$0.027 \pm 8.0 \cdot 10^{-4}$ b	$0.032 \pm 2.0 \cdot 10^{-4}$ b	$2.8 \cdot 10^{-4} \pm 8 \cdot 10^{-6}$ c
250	$0.015 \pm 8.0 \cdot 10^{-4}$ b	$0.026 \pm 4.0 \cdot 10^{-4}$ c	$3.3 \cdot 10^{-4} \pm 2 \cdot 10^{-5}$ b

Means with different letters in each column indicate significant differences according to the Tukey test ($P \leq 0.05$) (n = 15), ± standard error.

Nenova (2006) reported that the RGR value was reduced 51.95% in plants subjected to applications of 40 mg L^{-1} Fe^{2+} 34 d after the induction of the toxicity, in comparison to plants with optimal Fe^{2+} applications (2 mg L^{-1}). Similarly, Snowden and Wheeler (1993), who worked with 43 plant species native to British swamps, found that high Fe^{2+} concentrations (10-100 mg L^{-1}) significantly reduced the RGR in all the species except two (*Eriophorum angustifolium* and *Juncus effusus*); furthermore, the same authors confirmed that the dicotyledonous plants were more susceptible to Fe^{2+} toxicity, possibly due to the fact that monocotyledons are generally more porous in the shoots, which facilitates the diffusion of oxygen towards the roots of the plants. Among the dicotyledons, six species reduced their RGR 60% when 10 mg L^{-1} Fe^{2+} was added, in comparison with the control plants, showing evidence of sensitivity of the dicotyledonous class to excess Fe^{2+} stress. Similarly, Oliveira-Jucoski *et al.* (2013) found that a high accumulation of iron in the plant tissues of *Eugenia uniflora*, induced by elevated concentrations of the metal in the substrate, produced a reduction in the RGR.

Kampfenkel *et al.* (1995) confirmed that the rate of photosynthesis in conditions of saturated CO_2 in leaves of *Nicotiana plumbaginofolia* decreased 40% after exposure to excess Fe^{2+} as a consequence of an increase in foliar Fe^{2+} content; which in turn caused a 25% decrease in the starch content of the studied leaves. In the present study, the NAR decreased in the plants subjected to excess Fe^{2+} and this variable is directly proportional to the rate of photosynthesis of the plants. The reduction in the synthesis of sucrose and starch in the leaves exposed to Fe^{2+} toxicity was accompanied by an inhibition of photosynthesis, an increase in reduced *A* quinones (Q_A) and high photochemical extinction coefficient values q_N (photochemical losses related to heat, pH gradient and photoinhibition), all under conditions of high radiation and with very few variations with low radiation (Neuhaus *et al.*, 1989; Neuhaus and Stitt, 1991). This increase in the reduced Q_A was the result of the low pool of the same and as a consequence of increased non-photochemical losses. This induces a low electron transport rate which, together with the ionic stress produced by the excess Fe^{2+}, causes a decrease in the synthesis of photoassimilates in the leaves of plants. However, in addition to a decrease in the rate of photosynthesis as a consequence of excess Fe^{2+}, a increase in the respiration rate of the stressed leaves is produced, which supposes a higher consumption or rupture of hexoses, for which the leaves subjected to high Fe^{2+} concentrations suffered a strong decrease in the contents of glucose and fructose in comparison with the controls (Kampfenkel *et al.*, 1995). This is supported by studies carried out by Peña-Olmos and Casierra-Posada (2013), who found an increase of initial fluorescence (F_0) in the same plants as the present study. This increase was possibly due to damage in the reaction centers of photosystem II (PSII) (Vieira *et al.*, 2010) or to a decrease in the transference of excitation energy from the antenna complex towards the reaction centers (Baker and Rosenqvist, 2004), which may have produced a decrease in the photoinhibition of PSII.

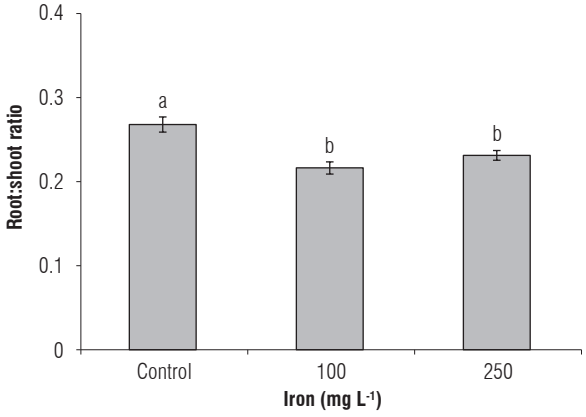

FIGURE 4. Root:shoot ratio in broccoli plants subjected to iron toxicity. Means with different letters indicate significant differences according to Tukey test ($P \leq 0.05$) (n=15). Error bars indicate standard error.

The root:shoot ratio presented significant differences between the treatments ($P \leq 0.05$). The addition of 100 and 250 mg L^{-1} Fe^{2+} to the substrate in which the broccoli plants grew induced a reduction of 19.29 and 13.72% in the value of this variable with respect to the control plants (Fig. 4).

Snowden and Wheeler (1993) suggested that plants can be classified as tolerant to excess Fe^{2+} by taking into account

the root:shoot ratio. In this way, these authors considered that if Fe^{2+} applications induced decreases in said ratio with respect to control plants, the plants should be considered sensitive to excess iron due to the fact that the root is more affected than the aerial part of the plant. A higher root:shoot ratio in tolerant plants is possibly due to fact that a high Fe^{2+} concentration in the substrate causes a higher accumulation of ochre deposits in the roots, which would increase the radicle mass. Similarly, Kampfenkel *et al.* (1995) suggested that under conditions of excess Fe^{2+}, it is separated and stored in the necrotic points that are developed in the leaves as a consequence of the same stress, as well as in the vacuoles of the mesophyll cells, with which the DW of the leaves would increase in relation to the roots of the plant, producing a decrease in the root:shoot ratio.

Conclusions

The analysis of variance carried out with the data obtained for the different parameters of the plant growth evaluation of the present study allowed for the conclusion that the iron toxicity produced stress in the evaluated plants, which affected the proportion and accumulation of DM in the different plant organs. The reduction in the leaf area expansion and in the evaluated growth indices evidenced a decrease in crop development. And so, it is evident that the exposure of *Brassica oleracea* var. *Italica* plants to high Fe^{2+} levels in the substrate negatively affects the general function of these plants.

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