Journal of Stress Physiology & Biochemistry, Vol. 9 No. 4 2013, pp. 39-52 ISSN 1997-0838

ORIGINAL ARTICLE

Differential Responses of Two *Lactuca sativa* Varieties to Bicarbonate-Induced Iron Deficiency.

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Received May 23, 2013

Iron chlorosis induced by bicarbonate is very common in calcareous soils, where bicarbonate (HCO_3^-) ions are present at high concentrations. In this study, morpho-physiological and biochemical responses of two *Lactuca sativa* varieties (Romaine and Vista) to bicarbonate-induced iron deficiency were investigated. The culture was conducted on nutrient solution containing 5 µM Fe and 10 mM $NaHCO_3$, in a growth chamber with controlled conditions. After 14 days of bicarbonate treatment, the two varieties seedling showed a slight yellowing of young leaves associated with a significant decline of plant biomass, leaf number and area. Furthermore, the concentrations of the nutrient elements (potassium, magnesium, iron and calcium) in leaves and roots of two lettuce varieties were modified. In roots of bicarbonate-treated plants, the Fe-chelate reductase activity was increased as compared to control in both varieties. PEPC activity was enhanced only in Vista variety. Moreover, Fe deficiency induced a small change in the photosynthetic parameters and chlorophyll fluorescence, especially in Romaine variety. These changes are accompanied by decreases in ribulose 1.5-bisphosphate carboxylase/oxygenase (Rubisco) activity. These findings indicated that vista variety could survive at low iron supply.

Key words: Lactuca sativa, bicarbonate, iron deficiency, photosynthetic activity.

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Key words: Lactuca sativa, bicarbonate, iron deficiency, photosynthetic activity.

Iron is an essential nutrient for plants. The major metabolic function of this mineral is electron transfer reaction. As a transition element, Fe exists in two oxidative states: ferrous (Fe^{2+}) and ferric (Fe^{3+}). For this reason, it is required for a wide range of biological functions like electron-transport chains of plant, photosynthesis and respiration (Briat and Vert 2004). Fe deficiency chlorosis is a common abiotic stress affecting plants in many areas of the world (Victor Del Rio et al. 2008), particularly in calcareous soils. Indeed, one third of the cultivated area in the world is made of calcareous soils and is

considered as Fe-deficient lands (Mori 1999). In such soils, bicarbonate has been regarded to as a major factor inducing Fe deficiency (Mengel 1994). In fact, HCO_3^- anions impair both Fe uptake by roots and its translocation to different organs in the plants (Römheld and Marschner 1986). In general, Fe deficiency chlorosis is associated with high pH in calcareous soils and low soil Fe availability (Chen *et al.* 2007). Consequently, iron deficiency is among the major problems affecting species yield in the country (Ksouri *et al.* 2006).

In these adverse conditions, dicotyledons and non graminaceous monocotyledons plants have developed physiological and biochemical responses, in particular at the root level, in order to increase iron availability and uptake by plants (Marschner and Romheld 1995). The main response is the increased activity of a trans-plasma membrane NAD(P)H dependent Fe(III)-chelate reductase (FCR), since the reduction of Fe^{3+} to Fe^{2+} is an obligatory step for Fe uptake (Chaney *et al.* 1972). Moreover, hydrogen ion excretion, due to the activity of the plasma membrane H^+-ATPase, increases the solubility of Fe compounds and provides an optimal pH value at the root / soil interface for the FCR activity (Zocchi and Cocucci 1990).

Lettuce (*Lactuca sativa L.*) is an important leafy vegetable mainly consumed fresh in salads and a good dietary source of phytochemicals, such as phenolic compounds and vitamins A, C, and E as well as minerals, such as calcium and iron, which are essential in preventing diseases and promoting health and wellness in people (Caldwell 2003). Previous studies have demonstrated the impact of different production environments on phytochemical antioxidants in lettuce (Zhao *et al.* 2007). It is considered to be a convenient model crop to investigate the relationship among light stress tolerance, photoacclimatory plasticity, and nutritional quality of vegetables.

The aim of the current research is to assess the variability of tolerance of two lettuce varieties (Romaine and Vista) cultivated under Fe deficiency (HCO_3^- induced) conditions by comparing physiological and photosynthetic parameters.

MATERIALS AND METHODS

Plant material and growth conditions

Lettuce (*Lactuca sativa*) seeds from cultivars Romaine and Vista were germinated and grown in pots containing a mixture of sand and peat 1:2 (v/v). After 7 days, seedlings were transferred in plastic pots of 300 ml in the same conditions of culture. Seedlings were irrigated with a complete nutritive solution contained 5 µM Fe-EDTA during 14 days in a growth chamber under controlled conditions (12 h daily/light, photon flux density 150 µmol m^{-2} s^{-1}, temperature 22/18°C; relative humidity 60/80% day/night). After 3 weeks, plants were divided into two groups. In the first one, seedlings were cultivated in the same nutrient solution with 5 µM Fe-EDTA, as a control. In the second, seedlings were maintained under the same conditions (5 µM Fe) with adding bicarbonate (10 mM). The pH of nutrient solution was buffered at 7.7 by adding NaOH (1N). The plants were harvested after 15 days of treatment and fresh and dry weight were determined.

Nutrient extraction and analysis

Mineral nutrients were extracted following the method of Liorente *et al.* (1976). Shoots and roots (8 plants per treatment) were washed with distilled water, dried at 70 °C for 72 h, weighed then ground with a mortar. The obtained powder samples were digested using 1N HCl solution. Iron, magnesium and calcium concentrations were determined by

atomic absorption spectrophotometry (VARIAN 220 FS). Potassium was measured by flame emission spectrophotometer (Corning photometer).

Leaf chlorophyll concentration

Chlorophyll concentration of young leaves was determined according to the method of Torrecilas *et al.* (1984). Five millilitre of pure acetone were added to fresh leaf samples. The total extraction took place after 72 h in darkness at 4°C. Extract absorbance were measured at 649 and 665nm.

Determination of Fe(III) reduction capacity by roots

Fe(III)-reductase activity was measured in roots by using bathophenanthrolinedisulphonate (BPDS) (Chaney *et al.* 1972). Excised root (1g from apical region) of 6 plants of each treatment were placed in a bottle filled with 5 ml of an assay solution consisting of: 0.1 mM Fe(III)-EDTA, 0.3 mM BPDS and 10 mM MES at pH 6 adjusted by NaOH, 1M. The bottles were kept in the dark at 25 ºC. After 30 minutes 2 ml of the solution were withdrawn and the absorbance at 535 nm was determined spectrophotometrically. The amount of reduced Fe was calculated by the concentration of the formed Fe(II)- (BPDS) complex, using an extinction coefficient of 22.14 $mM^{-1}.cm^{-1}$.

Enzyme extraction and assay

Fresh leaf or root samples were ground in a mortar with 100 mM Tris–bicine (pH 8.0) containing 1 mM EDTA, 5% glycerol (v/v), 5 mM $MgCl_2$, 1% mercaptoethanol (v/v), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 5% polyvinylpyrrolidone (PVP) (w/v of sample FW). After centrifugation at 12,000 g for 20 min at 4°C, the supernatant was collected and enzyme activities were measured immediately.

The activities of phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) and Rubisco (Rubisco E.C.4.1.1.39) were assayed according to Ouerghi *et al.* (2000). PEPC reaction mixture contained 100 mM Tris–bicine (pH 8.0), 5 mM $MgCl_2$, 1 mM DTT, 5 mM $NaHCO_3$, 0.2 mM NADH and 4 mM phosphoenolpyruvate. The crude extract was added to the reaction medium then the activity was monitored at 340 nm, for 15 min.

Rubisco activity was assayed in a reaction medium containing 100 mM Tris–bicine (pH 8.0), 10 mM $MgCl_2$, 0.2 mM EDTA, 5 mM dithiothreitol (DTT), 40 mM $NaHCO_3$, 4 mM ATP, 0.2 mM NADH, 0.2 mM ribulose 1,5-biphosphate (RuBP), 3-phosphoglycerate kinase (PGK) and glyceraldehyde 3-phosphate dehydrogenase (3-PGADH). The reaction was initiated by adding 0.2 mM RuBP, and activity was assayed spectrophotometrically at 340 nm, for 10 min at 30°C (Sato *et al.* 1980). Enzyme activities were expressed as $\mu mol\ h^{-1}\ g^{-1}$.

Photosynthetic parameters and chlorophyll fluorescence

At the end of the experiment, net assimilation rate of CO_2 (A), transpiration (E), stomatal conductance (gs) and intercellular CO_2 concentrations (Ci) were measured in fully expanded leaves of all plants using the ADC LCpro+ (Li-cor, LI- 6400XT, USA) portable measuring system. The chlorophyll fluorescence parameters were recorded parallel to the photosynthetic parameters, after a dark adaptation time for 30 min. Dark-adapted, maximum PSII efficiency was calculated as Fv/Fm. Actual (PSII) efficiency was calculated as (Fms–Fs)/Fms. Photochemical quenching (qP) was calculated as (Fms – Fs)/(Fms-F0). Non-photochemical quenching (NPQ) was calculated as (Fm/Fms) – 1 (Larbi et al. 2006).

Statistical analysis

Statistical analysis performed with Statistica™

software, using two-way ANOVA and Newman-Keuls test for post-hoc mean comparison. The ANOVA was performed over the whole set of data.

RESULTS

Iron deficiency symptoms

At harvest (14 days after the treatment), both varieties cultivated in nutrient solution containing bicarbonate (induced Fe-deficiency) showed the typical symptoms of iron-deficiency on their young leaves when compared with control plants. The earliest leaf chlorosis symptoms appeared in leaves of Romaine variety (about 3 days after the treatment).

Biomass production and leaf area expansion

Under Fe deficiency induced by bicarbonate, shoots biomass production in both varieties was significantly reduced as compared to the control (Table. 1). In Romaine, leaf dry weight was more reduced than that of Vista (66 and 43% less than the control, respectively). The harmful effect of bicarbonate on biomass was less pronounced in roots than in leaves. Leaf area expansion was also reduced by iron deficiency in both varieties with a more pronounced effect in Romaine than in Vista (about 68 and 48%, respectively). This reduction, mainly, associated with a reduction of leaf number (27% in Romaine and 20% in Vista) (Table. 1).

Nutrient content

Results of nutrient elements contents in two lettuce varieties, after 14 days of treatment, were summarized in Table 2.

Calcium

In Romaine leaves, calcium content was not significantly affected by iron deficiency; however, it was increased by 51% in roots. In Vista leaves and roots, calcium content was increased by 21 and 26%, respectively, under Fe-deficiency conditions.

Potassium

Under Fe deficiency accumulation of K^+ was observed in Romaine leaves and roots as compared to the control (about 30 and 12%, respectively), but K^+ content in Vista tissues was reduced in the presence of 10 mM $NaHCO_3$ in nutrient solution (about 30 and 34%, respectively).

Magnesium

Under Fe deficiency magnesium content in roots was diminished in both varieties as compared to the control, by 33% and 40% respectively in Romaine and Vista; however magnesium content was not significantly affected by iron deficiency in leaves tissue.

Iron

With 10 mM bicarbonate, leaf and root iron contents in both varieties were significantly reduced as compared to the control, in which they were diminished by 40 and 21% respectively in Romaine leaves and roots and by 38 and 32% respectively in Vista leaves and roots.

Effects of Fe deficiency on photosynthetic parameters

Chlorophyll fluorescence

A significantly decrease in F_v/F_m values, that reflects the quantum yield of PSII, was found with Fe deficiency, only in Romaine leaves. In control leaves, F_v/F_m was higher than 0.78 for both varieties. At the end of the treatment period, F_v/F_m ratio decreased to 0.76 (Fig 1). Iron deficiency did not cause any sustained decrease in actual (PSII) efficiency in both varieties. In the same way, qP and NPQ were not affected by iron deficiency.

Photosynthetic parameters

Fe deficiency caused major decreases in photosynthetic rates (A) in Romaine (45%) and did

not decrease photosynthetic rates in Vista. Furthermore, no significant difference was found among the Fe deficiency regarding the intercellular CO_2 concentration (Ci) in both varieties. Stomatal conductance (Gs) was decreased by Fe deficiency only in Romaine leaves. Iron chlorosis decreased transpiration in Romaine leaves by 29.51%, however it increased transpiration in Vista leaves by 35% as compared to control (Fig 2).

Chlorophyll contents

To describe the chlorotic status of the leaves, their chlorophyll concentration was determined in young leaves (Table. 3). On the control medium, leaf total chlorophyll concentration in the two varieties varied during the culture period. It considerably decreased as compared to the control (approximately -82% in Romaine young leaves and -71% in Vista one), after 14 days of treatment. Under iron deficiency, total carotenoids were significantly reduced in both varieties as compared to the control (about 84% in Romaine and 78% in Vista) (Table. 3).

Rubisco activity

In Romaine, Rubisco activity was more reduced than that of Vista (69 and 49% less than the control, respectively) (Fig. 3).

Root biochemical responses to iron deficiency

Iron reduction activity

Fe(III)-reductase activity of excised roots was low in control plants of both varieties (0.86 to 0.94 nmol min^{-1} g^{-1} FW). This activity was markedly stimulated in Vista under indirect Fe-lowering availability (up to ca. 4.2-fold higher than the control), while no significant changes were recorded in Romaine (Fig 4).

PEPC activity

Similar values were found in control plants of both Romaine and Vista. At 10 mM NaHCO3, PEPC was ca. 5.4-fold higher in Vista as compared to the control (Fig 4), while no changes occurred in the sensitive variety Romaine.

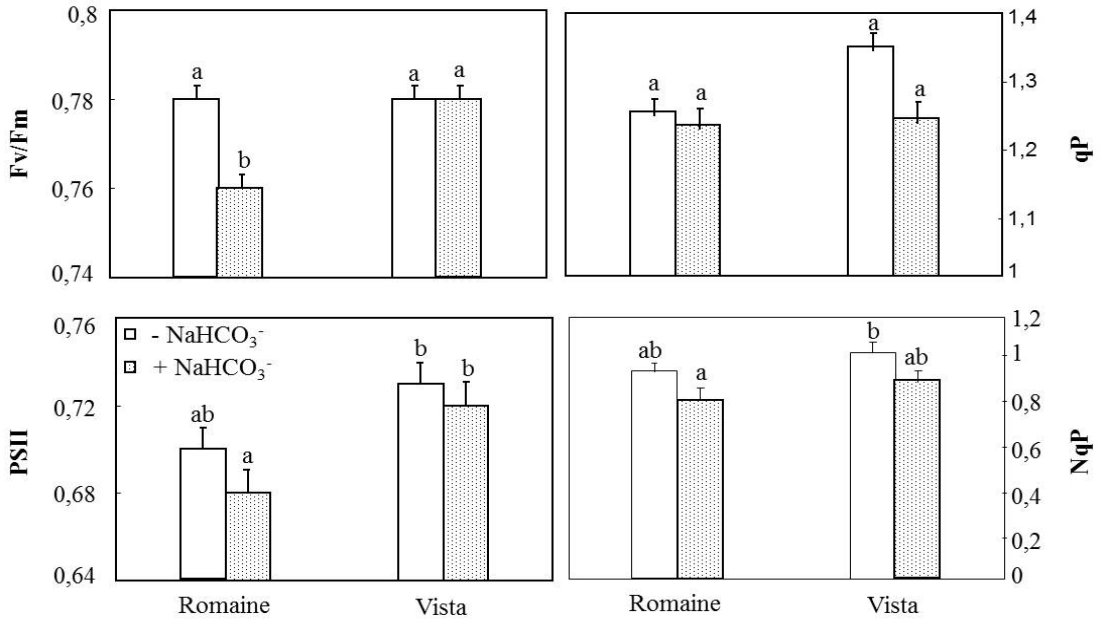

Figure 1. Chlorophyll fluorescence parameters of two lettuce varieties grown during 14 days on a control nutrient solution (-HCO_3^-) or containing 10 mM of bicarbonate (+HCO_3^-). Data are means of 8 replicates. Bars labelled by the same letter are not different according to the ANOVA test at $P \leq 0.05$.

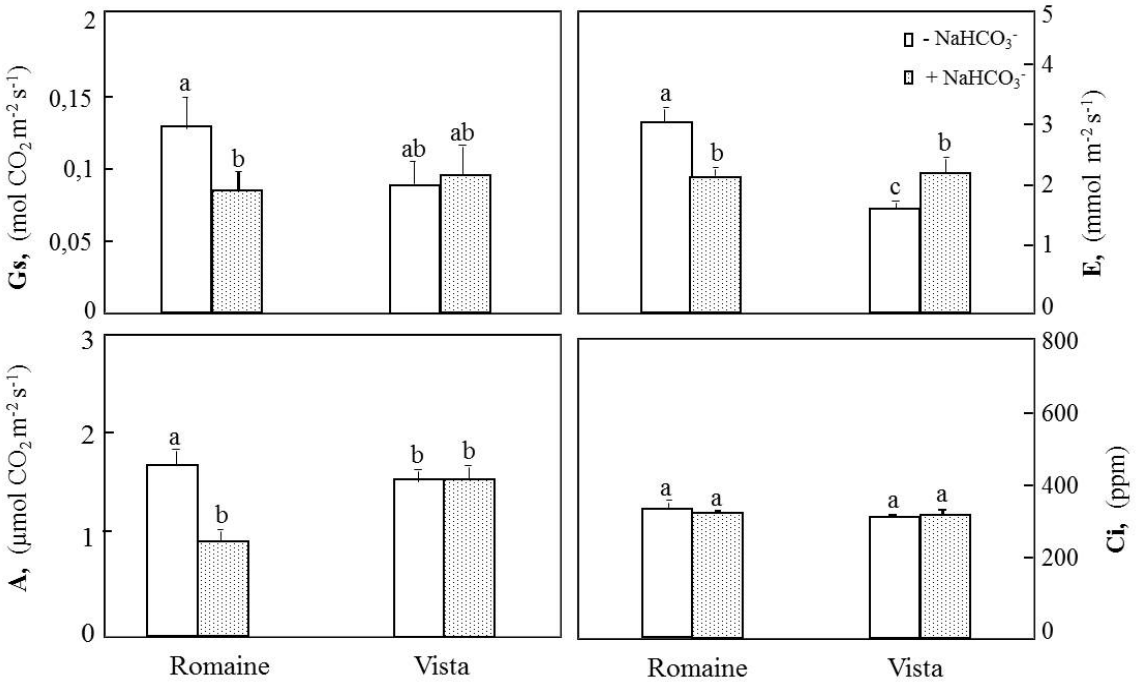

Figure 2. Stomatal conductance (Gs), CO_2 substomatal concentration (Ci), Net Photosynthesis (A) and transpiration rate (E) in leaves of two lettuce varieties grown during 14 days on a control nutrient solution (-HCO_3^-) or containing 10 mM of bicarbonate (+HCO_3^-). Data are means of 8 replicates. Bars labelled by the same letter are not different according to the ANOVA test at $P \leq 0.05$.

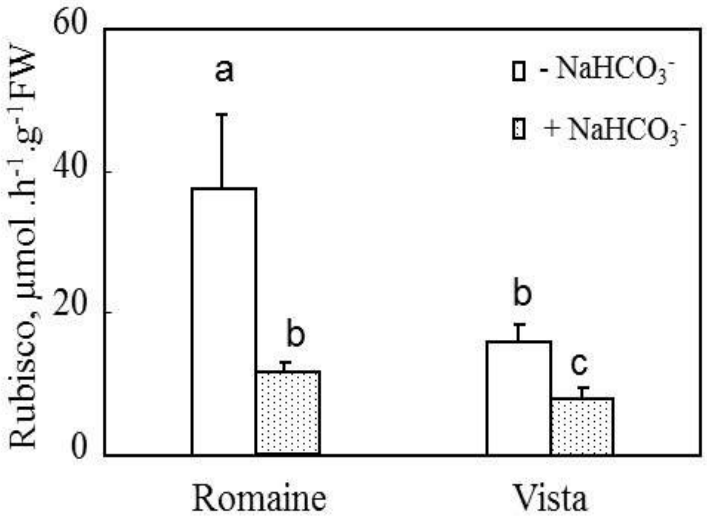

Figure 3. Rubisco activity in two lettuce varieties grown during 14 days on a control nutrient solution (-HCO_3^-) or containing 10 mM of bicarbonate (+HCO_3^-). Data are means of 3 replicates. Bars labelled by the same letter are not different according to the ANOVA test at $P \leq 0.05$.

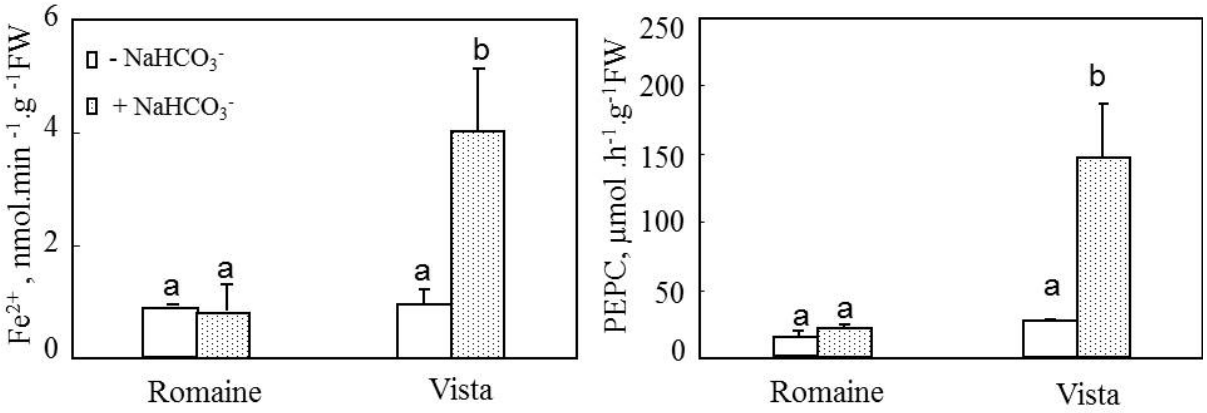

Figure 4. Fe-chelate reductase and PEPC activities in two lettuce varieties grown during 14 days on a control nutrient solution (-HCO_3^-) or containing 10 mM of bicarbonate (+HCO_3^-). Data are means of 6 replicates. Bars labelled by the same letter are not different according to the ANOVA test at $P \leq 0.05$.

Table 1. Plant growth parameters of two lettuce varieties grown during 14 days on a control nutrient solution (-HCO_3^-) or containing 10 mM of bicarbonate (+HCO_3^-). Data are means of 8 replicates. Those labelled by the same letter are not different according to the ANOVA test at $P \leq 0.05$.

	Romaine		Vista	
	-HCO_3^-	+HCO_3^-	-HCO_3^-	+HCO_3^-
Root biomass (mg)	28.62±3.84a	28.25±8.02a	64.32±13.3b	57.76±12.68b
Shoot biomass (mg)	333.63±59.9a	144.82±28.5b	651.3±91.9c	372.78±77.4a
Total leaf area (cm^2)	216.9±88.76a	68.5±13.21b	608±61c	315±76d
Leaf number	7.38±0.62a	5.38±0.89b	15±1c	12±2d

Table 2. Leaves and Roots Ca^{2+} , K^+ , Mg^{2+} and Fe^{2+} contents (µg.mg^{-1} DW) in two lettuce varieties after 14 days of growth on a control nutrient solution (0 mM HCO_3^-) or containing 10 mM of bicarbonate (+HCO_3^-) . Data are means of 8 replicates. Those labelled by the same letter are not different according to the ANOVA test at $P \leq 0.05$.

	Romaine				Vista			
	Leaves		Roots		Leaves		Roots	
	0	10	0	10	0	10	0	10
Ca^{2+}	12.3±2.6a	10.2±1.5a	58.5±9.9b	88.5±7.1c	16.0±0.8d	19.4±1.7d	31.0±8.5e	39.0±8.1e
K^+	54.5±1.6a	71.0±6.0b	100.0±16.7c	112.2±20.0c	81.5±5.2b	56.7±8.1a	275.0±36.3d	182.3±46.1e
Mg^{2+}	1.77±0.2a	1.42±0.2a	12.2±1.7b	8.06±2.3c	2.9±0.3d	2.8±0.4d	8.12±2.8c	4.9±1.03e
Fe^{2+}	0.20±0.02a	0.12±0.02b	5.20±1.0c	4.11±0.3c	0.24±0.03a	0.15±0.02b	1.42±0.04d	0.97±0.25d

Table 3. Leaf pigment concentrations (mg.g^{-1} FW) in two lettuce varieties after 14 days of growth on a control nutrient solution (-HCO_3^-) or containing 10 mM of bicarbonate (+HCO_3^-). Data are means of 6 replicates. Those labelled by the same letter are not different according to the ANOVA test at $P \leq 0.05$.

	Romaine		Vista	
	-HCO_3^-	+HCO_3^-	-HCO_3^-	+HCO_3^-
Total Chl	1.45±0.35a	0.264±0.074b	0.661±0.127c	0.193±0.087b
Total Car	0.302±0.081a	0.049±0.019b	0.14±0.035c	0.030±0.015b

DISCUSSION

In this work, the response of two varieties of *Lactuca sativa* to bicarbonate induced iron deficiency was investigated. Our results showed that bicarbonate induced iron deficiency chlorosis symptoms. Leaf chlorosis symptoms are important criteria in the evaluation of iron deficiency effects. In addition, bicarbonate inhibited shoot growth of both varieties with a more pronounced effect in Romaine than in Vista, when expressed as biomass production or as leaf number and leaf surface area. Roots, however, were much less sensitive to bicarbonate treatment than leaves, showing no significant variation between control and treated plants. These results are in agreement with those of Msilini *et al.* (2009). As documented in several other species (Molassiotis *et al.* 2006; Ksouri *et al.* 2007; Zocchi *et al.* 2007), Fe deficiency adversely affected plant growth activity, including the whole plant DW, leaf number, and individual leaf biomass.

Fe^{2+} content was more reduced in aerial organs of Romaine than in those of Vista, which could explain the more pronounced decrease in biomass production in the former. However, its content in roots of Vista was more reduced than in those of Romaine. According to these data, it appears that Vista is more able to transport iron towards its aerial parts than Romaine.

Gharsalli and Hajji (2002) observed that iron uptake by peach roots was not significantly decreased in the presence of HCO_3^-, but that transport towards aerial organs was strongly inhibited.

Our findings showed also that iron deficiency increased shoot and root potassium content only in Romaine; whereas it decreased that of magnesium in both varieties and it decreased that of calcium only in Romaine leaves. According to Tagliavini and Rombola (2001), K^+ plays a major role in Fe assimilation under iron deficiency conditions, by increasing root plasma membrane H^+-ATPase activity. Thus, K^+ accumulation in leaves may be related to the tolerance of this constraint (Szlek *et al.* 1990). Celik *et al.* (2006) observed that potassium, magnesium and calcium contents on both shoots and roots of maize varieties, degreased by the application of both Fe-EDTA and $NaHCO_3$.

Chlorophyll fluorescence analysis is usually an excellent tool to study photochemical damage. Morales *et al.* (2001), however, pointed out that the maximal yield of fluorescence in dark-adapted leaves (Fv/Fm) may not indicate real damage of PSII in iron-deficient leaves. These authors demonstrated that iron deficiency may also disconnect PSII antennae from reaction centres, and that fluorescence may also be released from disconnected PSII antennae.

However, the fluorescence maximal yield of dark-adapted leaves was reduced by the present treatment only in Romaine. This decrease of

maximal fluorescence likely reflects the inhibition of oxygen evolution and inability to provide electrons to adequately reduce the acceptor side of PSII. Both chlorophyll fluorescence parameters (Fv/Fm and Fv/F_0) decreased in the Fe-deficient leaves of Cadaman as well as in the bicarbonate-treated leaves of GF-677 (Molassiotis *et al.* 2006). Similar observations were previously reported some higher plants (Morales *et al.* 1991; Larbi *et al.* 2006; Timperio *et al.* 2007) affected by iron deficiency. The loss of PSII photochemical activity has been attributed to a decrease in the photochemical capacity of PSII pigments as iron is required for their biosynthesis (Morales *et al.* 1991).

Bicarbonate treatment leads to a significant decrease of net CO_2 assimilation rate (*A*), only in Romaine in concomitance with a decrease of the transpiration rate and the stomatal conductance (*gs*). In Vista leaves, conductance (*gs*) was not affected, however, transpiration rate increased by bicarbonate. On the other hand, *Ci* was either unchanged in the two varieties in response to Fe deficiency. Contrasting to previous studies, our data suggest that photosynthesis activity can be affected by stomatal component. Molassiotis *et al.* (2006), showed that in the Fe-deficient leaves of Cadaman and in the bicarbonate-treated leaves of GF-677 the intercellular CO_2 concentration was not affected while at the same time both photosynthetic rate (Pn) and gs decreased significantly. According to Yakushiji *et al.* (1998), these data imply that the decrease in the Pn has been caused not only by stomatal closure (stomatal limitations), but also by a decrease in carboxylation efficiency (non-stomatal limitations). Larbi *et al.* (2006) reported that Fe deficiency was more inhibitory on photosynthetic rates than on transpiration rates, resulting in lower water use efficiency. On the other hand, the internal CO_2 concentration (*Ci*) remained high or even increased in response to Fe deficiency. According to these authors, the main cause for the lower rates of photosynthesis under Fe deficiency in sugar beet could be the reduction of stomatal opening.

Low photosynthetic rates could also be associated with an inefficient Rubisco activity, one of the major enzymes of the carbon fixation cycle. The activity of Rubisco, was more declined in Romaine than that of Vista plants exposed to Fe deficiency. Similar reduction in the activity of this enzyme was shown by Bertamini *et al.* (2001) in Fe-deficient grapevine. Larbi *et al.* (2006) reported also a decline in Rubisco activity with severe Fe deficiency in sugar beet, pear and peach. Msilini *et al.* (2009) showed, indeed, that bicarbonate-induced Fe deficiency diminished photosynthetic competence by lowering RuBP carboxylation capacity through a reduction of leaf Rubisco activation. The lower RuBP carboxylation capacity was not previously attributed to reduced activation of the Rubisco enzyme (Taylor and Terry, 1984), but rather to a down-regulation of gene expression, resulting in RuBP levels reduced by 70%. Biochemical damage leading to a reduction in CO_2 capture by Rubisco may therefore also play a role in inhibiting photosynthesis of iron-deficient leaves, even when light is not a limiting factor. According to Arulanantham *et al.* (1990), the decrease in photosynthesis with Fe deficiency mediated reduction in photochemical capacity was most likely mediated through a reduction in RuBP regeneration (as opposed to a decrease in rubisco activity).

The photosynthesis inhibition in bicarbonate-treated plants could be ascribed to the strong reduction of the chlorophyll concentration. In our study, a decrease in leaf total chlorophyll content

and leaf total carotenoid contents was observed in both varieties exposed to bicarbonate treatments. This decline was more pronounced in Romaine leaves. Our results show that Fe deprivation-induced Fe chlorosis of the young leaves. These visual symptoms resulted from the significant decrease of chlorophyll concentration in these organs since Fe is implied in the synthesis of chlorophyll and carotenoid (Abadia and Abadia, 1993; Pestana *et al.* 2001). On the other hand, several studies demonstrate that Fe deficiency stimulate the chlorophyllase, enzyme implied in the degradation of chlorophyll, in citrus (Ferrnandez-Lopez *et al.* 1991) and barley (Rodriguez *et al.* 1987). Such a large decrease in the chlorophyll content would be ascribed to the role of Fe inte the formation of *d*-aminolevulinic acid and protochlorophyllide, the precursors of the chlorophyll biosynthesis (Marschner, 1995). On the other hand, the reduction of chlorophyll contents was accompanied by a decline of the maximum quantum yield of PSII (Fv/Fm) in Fe deficient leaves of sugar beet (Morales *et al.* 1991) and field grown peach trees (Nedunchezhian *et al.* 1997).

Iron uptake efficiency of strategy I plants have been largely attributed to the adaptive biochemical mechanisms developed at root cell plasma membrane. Acidification capacity and Fe(III)-chelate reduction by Fe-chelate reductase have been shown to be stimulated in response to Fe deficiency (Schmidt 2006; Zaharieva *et al.* 2004). We did not observe root acidification under bicarbonate constraint, probably because of the bicarbonate buffer power. A similar result was found by M'sehli *et al.* (2009a), in the presence of bicarbonate, the pH of the nutrient solution was not significantly modified by both ecotypes of *Medicago ciliaris*. Molassiotis *et al.* (2006) suggested that the rhizosphere acidification process of Strategy I plants is possibly inhibited by HCO_3^-.

However, Fe-chelate reductase activity was significantly increased in bicarbonate-treated Vista plants, suggesting that only Vista was able to reduce and thus to absorb Fe despite the presence of HCO_3^- ions. This induction of root FCR activity was correlated to the tolerance to Fe deficiency in several species, as it was reported for Kiwifruit (Rombolà *et al.* 2002), Chickpea (Mahmoudi *et al.* 2007), Grapevine (Ksouri *et al.* 2006) and *Medicago* (M'sehli *et al.* 2009a), Kelvedon (Jelali *et al.* 2010). In the same way, an important root PEPC ability was registered in the tolerant varieties (Vista) treated with bicarbonate, in contrast to Romaine. Ollat *et al.* (2003) indicated that this enzyme may control organic acid biosynthesis and accumulation in Fe-shortage. These findings suggest that the induction of FCR and PEPC activities in roots in response to Fe deficiency constitutes an important physiological adaptation enabling Vista plants to tolerate the stress. In the same way, and in response to Fe deficiency, Rombolà *et al.* (2002) showed that the tolerant genotype of Kiwifruit showed a higher FCR activity associated with a higher PEPC activity compared to the sensitive genotype.

CONCLUSIONS

On the basis of our comparative analysis, Vista variety was found more resistant to the harmful effects of iron deficiency as compared to Romaine. This lower sensitivity was due, not only to the light colour of Vista leaves, but also, to the maintenance of biosynthetic activity, nutritional status and a better enzymatic activities in roots.

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