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ORIGINAL PAPER

Role of arbuscular mycorrhizal symbiosis in root mineral uptake under $CaCO_3$ stress

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Abstract This study investigated the effects of increasing $CaCO_3$ concentrations (0, 5, 10, 20 mM) on arbuscular mycorrhizal (AM) symbiosis establishment as well as on chicory root growth and mineral nutrient uptake in a monoxenic system. Although $CaCO_3$ treatments significantly decreased root growth and altered the symbiosis-related development steps of the AM fungus *Rhizophagus irregularis* (germination, germination hypha elongation, root colonization rate, extraradical hyphal development, sporulation), the fungus was able to completely fulfill its life cycle. Even when root growth decreased more drastically in mycorrhizal roots than in non-mycorrhizal ones in the presence of high $CaCO_3$ levels, the AM symbiosis was found to be beneficial for root mineral uptake. Significant increases in P, N, Fe, Zn and Cu concentrations were recorded in the mycorrhizal roots. Whereas acid and alkaline phosphatase enzymatic activities remained constant in mycorrhizal roots, they were affected in non-mycorrhizal roots grown in the presence of $CaCO_3$ when compared with the control.

Keywords $CaCO_3$ · *Rhizophagus irregularis* · Arbuscular mycorrhizal symbiosis · Mineral uptake · Phosphatase

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Introduction

Calcareous soils, characterized by the presence of high levels of total and active calcium carbonate ($CaCO_3$), are common in arid and semi-arid climates (Leytem and Mikkelsen 2005). They represent 30% of total land areas (Chen and Barak 1982). Phosphorous (P) deficiency is often observed in plants cultivated on calcareous soils, and it induces a decrease in crop yields without detectable symptoms in many cases (Hopkins and Ellsworth 2005). In calcareous soils, P is fixed by calcium carbonate ($CaCO_3$) through adsorption and precipitation that gradually decreases its solubility and consequently its availability to plants. Also, iron (Fe) (Lindsay 1995; Bavaresco et al. 2003; Ksouri et al. 2005), manganese (Mn) (Lucas and Knezek 1972) and zinc (Zn) (Saeed 1977) deficiencies are often observed in calcareous soils. In fact, $CaCO_3$ impairs Fe and Mn plant absorption by oxidation of Fe^{2+} ions and precipitation and adsorption of Mn^{2+} ions (Le Tacon 1978).

In natural ecosystems, plant species can be categorized according to their ability to grow in calcareous soils: calcicole plants are found in calcareous soils and the calcifuge plants are unable to tolerate calcareous soils (Lapeyrie 1990). The difference in the ability of plants to grow on calcareous soils could be explained by the physiology of their roots in the acquisition of nutrients (Gutschick 1993). The efficiency of mineral absorption depends on root size, morphology, physiology and biochemistry (Marschener 1995). Formation of proteoid (cluster) roots, enhancement of organic acid synthesis, secretion of acid phosphatase, H^+-ATPase (Vance et al. 2003) and activation of P transporters (Raghothama 1999) are among the numerous strategies of plant adaptations to P deficiency. In the presence of Fe deficiency, several plant species are able to produce phytosiderophores as metal ion

transporters to improve Fe absorption (Romheld 1987; Hell and Stephan 2003).

The arbuscular mycorrhizal (AM) symbiosis is also a plant survival strategy in the presence of mineral nutrient deficiencies (Helgason and Fitter 2005). In fact, AM symbiosis increases the soil volume explored by the plant roots which is of fundamental importance to uptake of less mobile nutrients like P and Cu, especially in calcareous soils. The development of hyphal networks inside and around mycorrhizal roots (Lee and George 2005) is especially known for improving P and N uptake by plants (Johansen et al. 1993; Tobar et al. 1994; Smith and Read 2008). Several studies have demonstrated the implication of AM symbiosis in the increment of Fe, Zn and Cu uptake (Clark and Zeto 1996; Silvia et al. 2005; Schreiner 2007), especially under stressed conditions. Many mechanisms are supposed to be involved in the higher solubility of immobile or fixed minerals: production of some enzymes such as phosphatases (Marschner and Dell 1994) or siderophores by both AM fungi and roots (Caris et al. 1998; Haselwandter 2008). However, no study has reported the impact of high $CaCO_3$ levels on AM symbiosis establishment and on mycorrhizal root mineral uptake.

Thus, the aim of the current work was to study the effect of increasing concentrations of $CaCO_3$ (i) on the spore germination of the AM fungus *Rhizophagus irregularis* (formerly known as *Glomus irregularis*) and the establishment of the symbiosis (root colonization rate, extraradical hyphal development, sporulation), and (ii) on mineral nutrient uptake by the AM symbiosis under monoxenic conditions.

Materials and methods

Plant and fungal material

In this study, two experiments were undertaken. In the first one, spores were extracted from a 2-month-old monoxenic culture of Ri T-DNA transformed chicory (*Cichorium intybus* L.) roots colonized by *R. irregularis* (previously known as *Glomus irregulare* DAOM 197198, Schüßler and Walker 2010). One spore was placed in the middle of each Petri dish (5 cm) containing a modified M medium (Bécard and Fortin 1988) [solidified with 0,05% (ww/v) gellan gel (phytagel; Sigma, St. Louis, MO, USA)] supplemented or not (Control) with the different concentrations of $CaCO_3$ (0.5, 1 and 2 gl^{-1} equivalent to 5, 10 and 20 mM).

After the addition of $CaCO_3$ to obtain the concentrations cited before, media were sterilized (121°C, 30 min). The culture media were shaken to avoid the precipitation of $CaCO_3$ and distributed in sterile Petri dishes. Fifty spores per treatment were used.

The second experiment consisted in cultivating transformed chicory roots by *Agrobacterium rhizogenes* (Fontaine et al. 2004) colonized or not by *R. irregularis* on a modified M medium containing or not the same $CaCO_3$ concentrations as used in the first experiment. Cultures were inoculated from standardized root inoculum of 2-month-old monoxenic cultures of Ri T-DNA transformed chicory roots, colonized or not by *R. irregularis*, sampled using a 10-mm cork borer as described by Verdin et al. (2006). A disk of culture medium containing roots from monoxenic cultures (non-colonized or colonized chicory roots) was placed in the middle of each Petri dish (9 cm). After 8 weeks of culture, roots used as inoculum were discarded and were not taken into account in the different parameters analysed. During the two experiments, roots and spore cultures were incubated at 27°C in the dark.

Determination of *R. irregularis* spore germination and germination hypha development

The incubation period was 4 weeks. Spore germination was determined each 48 h during the first week and once a week until the end of the experiment. The germination hypha development was measured each week by observation under a microscope at 10× to 40× magnification using the gridline method, and data were transformed in the formula of Tennant (1975).

Determination of arbuscular mycorrhizal colonization

After 8 weeks of culture, extraradical mycelium density was measured with the same method used for the determination of the germination hypha length. The number of spores formed was counted (n=5 replicates) using the method described by Declerck et al. (2001). Roots were collected from the medium by solubilizing the solidified media for 15 min under agitation in 25 ml of Tris–HCl buffer (50 mM, pH 7.5) + EDTA (10 mM) (v/v), and filtering roots using a strainer. A first aliquot of roots collected from each replicate was cleared in KOH (10%) and stained with Trypan blue as described by Phillips and Hayman (1970) and modified by Koske and Gemma (1989) to determine root colonization by the method of McGonigle et al. (1990).

Determination of plant growth

Plant growth was estimated by measuring the length of mycorrhizal and non-mycorrhizal chicory roots. This parameter was assessed by the same technique used for the determination of the extraradical mycelium density, described above.

Determination of plant tissue and culture medium nutrient concentrations

Before nutrient extraction, chicory roots were oven dried for 72 h at 70°C. A portion of ground material (100 mg) from each replicate was incinerated during 5 h at 450°C. Ashes were collected in 20 ml of nitric acid (0.1 N) and digested at 100°C during 10 min. The digestions were filtrated, made up to 100 ml then stored at 4°C. These extractions were used to assay available phosphorous by the method of Olsen et al. (1954); exchangeable potassium, calcium, magnesium, manganese, zinc, iron and copper were determined using the methods described by Pauwels et al. (1992). Total nitrogen was determined in 100 mg of ground-dried roots with the Kjeldahl method (Jones 1991).

Culture mediums supplemented or not with increasing concentrations of $CaCO_3$ were placed on filter paper, and then oven-dried for 24 h at 70°C. Each dried medium was weighed then incinerated during 5 h at 450°C. The nutrient concentrations were estimated using the same methods applied for plant tissue.

Determination of alkaline and acid phosphatase activities

The second aliquot of the roots collected from each replicate was conserved at −80°C. A 100-mg portion of this aliquot was ground in 1 ml of STEB (sucrose triton extraction buffer, pH 8) as described by Sen and Hepper (1986). After centrifugation (20 min/16,000×*g*), supernatants were separated into three aliquots. Two aliquots were used to measure alkaline and acid phosphatase activities and the third one to determine total proteins concentrations. Phosphatase activities were assayed using the *p*-nitrophenylphosphate as the substrate (Wako LabAssay™ ALP; Wako Pure Chemical Industries, Osaka, Japan). This kit was adapted to evaluate each kind of phosphatase activity. Carbonate buffer (0.1 M, pH 8.8) was prepared as described by Delory and King (1945) to measure alkaline phosphatase activity. The method described by Green (1933) was adapted to prepare the sodium acetate buffer (0.1 M, pH 5.5) to measure acid phosphatase activity. Supernatants were added to 400 µl of each buffer containing the *p*-nitrophenylphosphate (6.7 mM) and incubated during 15 min in a water bath at 37°C. The enzymatic reaction was stopped by adding 320 µl of NaOH (0.2 M) to each sample. Activities were quantified by comparing the absorption at 405 nm to a standard curve of diluted *p*-nitrophenol solutions and NaOH. They were expressed in nmol $min^{-1} \mu g^{-1}$ of root proteins. Total protein concentrations were determined in supernatants using the Total Protein Kit, Micro Lowry, Peterson's Modification (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

Effects of $CaCO_3$ concentrations on the measured parameters were tested with a PROC ANOVA procedure of SAS (9.1) version with the LSD (Least Significant Difference $_{0.05}$) means comparison option. The percentage data (mycorrhizal colonization) were first arcsine transformed (Zar 1999).

Results

Impact of $CaCO_3$ on the AMF *R. irregularis* spore germination

$CaCO_3$ effects on *R. irregularis* spore germination were evaluated by counting the number of spores that germinated on media containing increasing concentrations of $CaCO_3$ (0, 5, 10, 20 mM) each 48 h during the first 8 days of incubation, and then once a week until 30 days.

Spore germination kinetic compared between the control medium and the medium supplemented with $CaCO_3$ showed significant differences (Fig. 1). Whereas about 68% of *R. irregularis* spores germinated rapidly during the first 2 days of incubation in the absence of $CaCO_3$ (control) (Fig. 1), spore germination was reduced to 36%, 12% and 28%, in the presence of 5, 10 and 20 mM $CaCO_3$, respectively. At 30 days, $CaCO_3$ led to lower germination percentages, estimated to be 40%, 40% and 50%, respectively, in comparison to the control, which reached 86% germination.

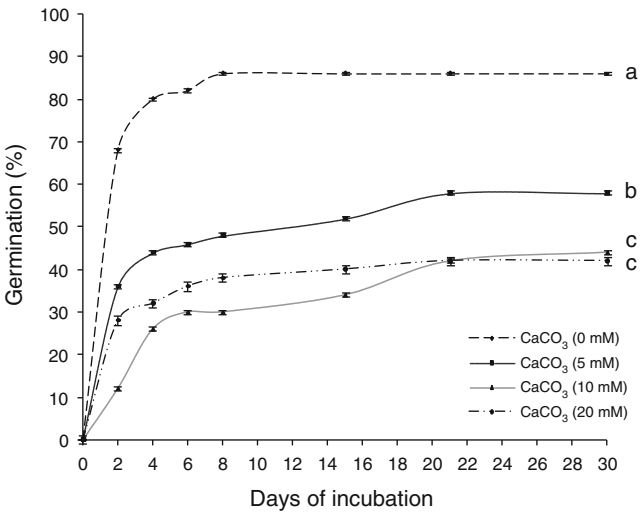

Fig. 1 Spore germination kinetic of *R. irregularis* during 30 days of incubation in the presence of increasing $CaCO_3$ concentrations (0, 5, 10 and 20 mM). Data are presented as means ± SE. Means were obtained from 50 replicates. Different letters indicate significant difference between spore germination kinetic plots according to the LSD test ($p<0.05$)

In the absence of $CaCO_3$ (control), germination hyphae reached a final length of 5.1 cm at 30 days of incubation (Fig. 2). In contrast, on $CaCO_3$ supplemented media, the rates of germination hyphal elongation were significantly slower and the maximum lengths reached at the end of the experiment were significantly ($p<0.05$) shorter than those of spores grown on control medium without $CaCO_3$. They were estimated to be about 1.6, 1.7 and 1 cm in media containing 5, 10, 20 mM $CaCO_3$, respectively.

Impact of $CaCO_3$ on the arbuscular mycorrhizal colonization

After 8 weeks of incubation, microscope observations of stained chicory roots showed that the percentages of colonization were significantly ($p<0.05$) higher in roots grown on control medium than on $CaCO_3$ supplemented media. Total colonization of the chicory roots was significantly reduced by 36%, 43% and 68% in the presence of 5, 10 and 20 mM of $CaCO_3$, respectively. Also, significant ($p<0.05$) decreases in arbuscule abundancy were observed in the presence of different $CaCO_3$ concentrations (5, 10, 20 mM). The arbuscule percentages were about 47%, 42%, 36% and 18% in the presence of 0, 5, 10, 20 mM of $CaCO_3$, respectively (Fig. 3).

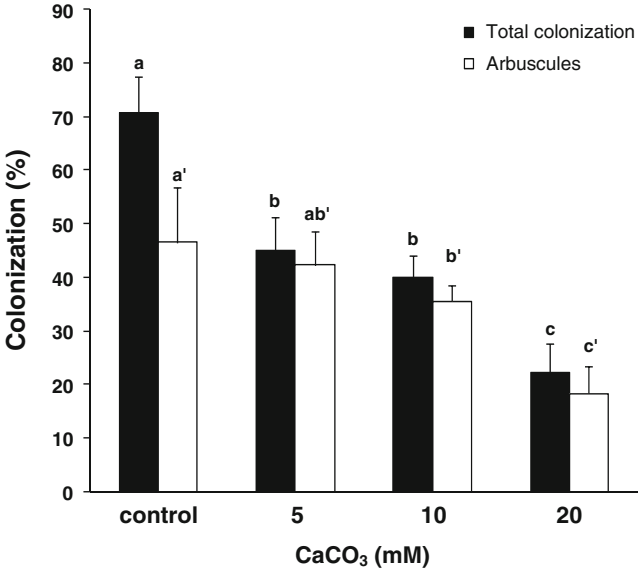

Fig. 3 Mycorrhizal colonization of transformed chicory roots (*Cichorium intybus* L.) colonized by *R. irregularis* after 8 weeks of growth in the absence and in the presence of increasing $CaCO_3$ concentrations. Data are presented as means ± SE. Means were obtained from five replicates. Different letters indicate significant differences between different $CaCO_3$ concentrations treatments according to the LSD test ($p<0.05$)

Impact of $CaCO_3$ on *R. irregularis* extraradical development

Extraradical hypha length decreased significantly ($p<0.05$) on $CaCO_3$ supplemented media. Whereas it was about 6.5 m in the absence of $CaCO_3$, values decreased to about 5, 1.7 and 0.4 m in the presence of 5, 10 and 20 mM of $CaCO_3$, respectively (Fig. 4).

Concerning sporulation, while no significant differences were observed in spore number on media containing 0, 5

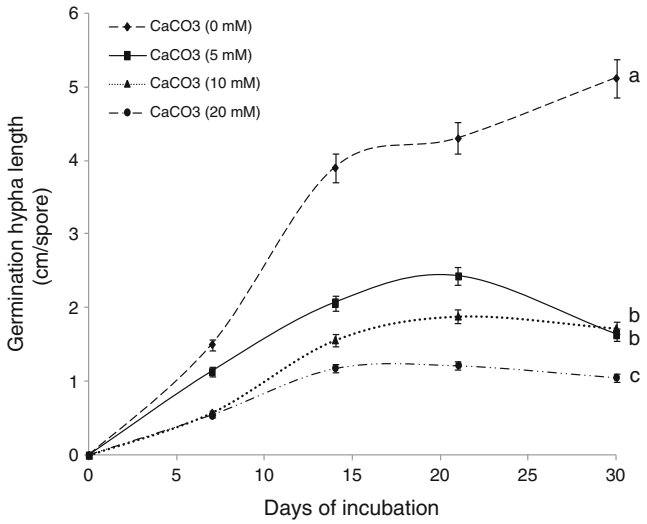

Fig. 2 *R. irregularis* germination hypha length during 30 days of incubation in the presence of increasing $CaCO_3$ concentrations (0, 5, 10 and 20 mM). Data are presented as means ± SE. Means were obtained from 43, 29, 22 and 21 replicates for 0, 5, 10 and 20 mM $CaCO_3$, respectively. Different letters indicate significant difference between the kinetic plots according to the LSD test ($p<0.05$)

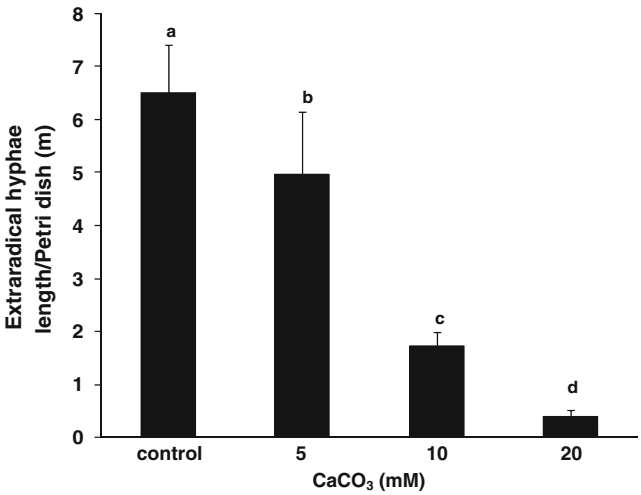

Fig. 4 Extraradical hyphae length of *R. irregularis* after 8 weeks of growth in the absence (control culture) and in the presence of increasing concentrations of $CaCO_3$. Data are presented as means ± SE. Means were obtained from five replicates. Different letters indicate significant differences between increasing concentrations of $CaCO_3$ according to the LSD test ($p<0.05$)

and 10 mM of $CaCO_3$, a drastic drop of 72% occurred on medium supplemented with 20 mM of $CaCO_3$ (Fig. 5).

Impact of $CaCO_3$ on chicory root growth

$CaCO_3$ effects on chicory root growth were determined by measuring root length after 8 weeks of incubation in the presence of increasing concentrations of $CaCO_3$ (0, 5, 10 and 20 mM). The highest chicory root length was about 6.6 m, and it was obtained with mycorrhizal roots in the absence of $CaCO_3$ (Fig. 6). Increasing concentrations of $CaCO_3$ in the culture medium significantly reduced ($p<0.05$) the length of mycorrhizal root by about 40%. However, no significant effect was observed on non-mycorrhizal roots (Fig. 6).

Impact of $CaCO_3$ on root mineral concentrations

Increases in medium pH, Ca, Mg and Mn concentrations were observed with increasing concentrations of $CaCO_3$ (Table 1). Cu, Zn and K concentrations increased only at 5 mM of $CaCO_3$. However, a decrease in N concentration was observed in particular at 5 mM of $CaCO_3$. No effect was observed on P concentrations in medium supplemented with $CaCO_3$ (Table 1).

In the absence of $CaCO_3$ (control), N, Mg, Mn and Cu concentrations were significantly higher in mycorrhizal roots compared to non-mycorrhizal ones, but no significant differences were observed between mycorrhizal and non-mycorrhizal roots for P, K and Zn concentrations (Table 2).

As $CaCO_3$ concentrations increased, N, P, Fe, Zn, Cu and Ca concentrations increased significantly in both mycorrhizal and non-mycorrhizal roots (Table 2). Mineral concentrations were higher in mycorrhizal roots compared to non-mycorrhizal ones, in most cases except for Ca. The highest N root concentration was observed in mycorrhizal roots at 20 mM of $CaCO_3$. P concentration was 3 times higher in mycorrhizal roots grown in the presence of $CaCO_3$ than in the control medium. The highest concentrations of Fe, Mn, Zn and Cu were obtained in mycorrhizal roots at 10, 20, 20 and 5 mM, respectively (Table 2). K concentration also increased significantly in mycorrhizal roots by 17% and 28% at 5 and 10 mM, respectively. However, in non-mycorrhizal roots, it decreased significantly ($p<0.05$) at 5 and 20 mM. Mg concentration was higher in mycorrhizal roots than in non-mycorrhizal ones, under $CaCO_3$ stress, despite the

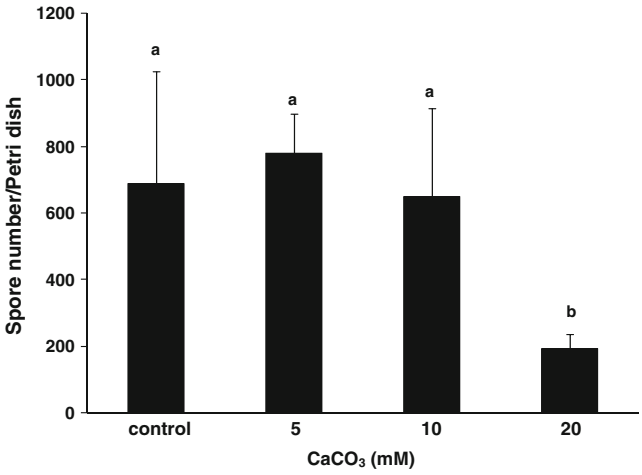

Fig 5 Spore number of *R. irregularis* after 8 weeks of growth in the absence or in the presence of increasing concentrations of $CaCO_3$. Data are presented as means ± SE. Means were obtained from five replicates. Different letters indicate significant differences between different concentrations treatments according to the LSD test ($p<0.05$)

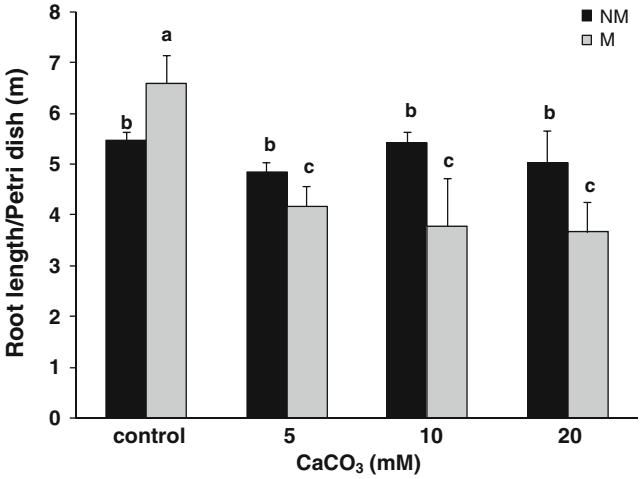

Fig. 6 Root length of transformed chicory roots (*C. intybus* L.) colonized or not by *R. irregularis* after 8 weeks of growth in the absence and in the presence of increasing $CaCO_3$ concentrations. Data are presented as means ± SE. Means were obtained from five replicates. Different letters indicate significant differences between non-mycorrhizal (*NM*) and mycorrhizal (*M*) roots with increasing $CaCO_3$ concentrations according to the LSD test ($p<0.05$)

Table 1 Medium nutrient concentration in the absence and in the presence of increasing concentrations of $CaCO_3$

$CaCO_3$ (mM)	0	5	10	20
pH	5.50	6.24	6.52	6.71
Medium nutrient concentration				
N (mg g^{-1} medium)	0.62	0.37	0.55	0.51
P (mg g^{-1} medium)	0.03	0.04	0.03	0.05
K (mg g^{-1} medium)	2.62	2.91	2.68	2.63
Ca (mg g^{-1} medium)	9.78	12.87	15.64	20.72
Mg (mg g^{-1} medium)	1.82	2.05	1.89	1.95
Fe (mg g^{-1} medium)	0.09	0.12	0.11	0.09
Mn (μg g^{-1} medium)	52.59	69.16	75.65	56.48
Zn (μg g^{-1} medium)	10.91	11.70	9.08	8.60
Cu (μg g^{-1} medium)	6.40	7.87	4.44	5.56

Table 2 Mineral concentrations in transformed chicory roots (*Cichorium intybus* L.) colonized (M) or not (NM) by *R. irregularis* after 8 weeks of growth in the absence and presence of increasing $CaCO_3$ concentrations

$CaCO_3$ (mM)	Control		$CaCO_3$					
Root nutrient concentrations			5		10		20	
	NM	M	NM	M	NM	M	NM	M
N (mg g^{-1} dry wt)	13.04 ± 0.11^{c}	15.68 ± 0.28^{b}	8.79 ± 0.77^{d}	15.26 ± 0.42^{b}	15.26 ± 0.09^{b}	18.40 ± 1.08^{a}	15.79 ± 0.84^{b}	19.18 ± 0.70^{a}
P (mg g^{-1} dry wt)	0.56 ± 0.00^{f}	0.57 ± 0.00^{f}	0.89 ± 0.08^{e}	1.58 ± 0.00^{a}	0.83 ± 0.00^{d}	1.24 ± 0.01^{b}	0.76 ± 0.00^{e}	1.22 ± 0.01^{b}
K (mg g^{-1} dry wt)	3.26 ± 0.05^{d}	3.36 ± 0.05^{d}	2.94 ± 0.13^{e}	3.93 ± 0.14^{c}	4.14 ± 0.12^{b}	4.31 ± 0.16^{a}	2.78 ± 0.11^{f}	2.66 ± 0.00^{f}
Ca (mg g^{-1} dry wt)	$24.66 \pm 0.57^{C,*}$	23.33 ± 0.57^{C}	$38.75 \pm 1.34^{B,}$	40.17 ± 1.48^{AB}	$43.70 \pm 1.28^{A,}$	42.15 ± 1.69^{A}	$40.13 \pm 1.17^{B,}$	38.88 ± 1.92^{B}
Mg (mg g^{-1} dry wt)	4.87 ± 0.00^{g}	5.65 ± 0.00^{a}	5.30 ± 0.02^{c}	5.48 ± 0.02^{b}	5.02 ± 0.02^{f}	5.25 ± 0.01^{d}	4.70 ± 0.01^{h}	5.15 ± 0.02^{e}
Fe (mg g^{-1} dry wt)	1.38 ± 0.00^{g}	1.31 ± 0.01^{h}	2.72 ± 0.02^{e}	2.91 ± 0.01^{c}	2.79 ± 0.01^{d}	3.54 ± 0.01^{a}	2.64 ± 0.01^{f}	3.35 ± 0.02^{b}
Mn (μg g^{-1} dry wt)	86.66 ± 5.77^{d}	260 ± 10.00^{b}	46.51 ± 0.00^{f}	256 ± 0.00^{b}	66.66 ± 0.00^{e}	147 ± 0.00^{c}	258 ± 11.78^{b}	522 ± 19.24^{a}
Zn (μg g^{-1} dry wt)	60 ± 0.00^{g}	60 ± 0.00^{g}	93.02 ± 0.00^{f}	154 ± 0.00^{c}	133 ± 0.00^{d}	176 ± 0.00^{b}	122 ± 0.00^{e}	200 ± 0.00^{a}
Cu (μg g^{-1} dry wt)	40 ± 0.00^{h}	50 ± 0.00^{e}	46 ± 0.00^{f}	76 ± 0.00^{a}	44 ± 0.00^{g}	58 ± 0.00^{d}	61 ± 0.00^{c}	66 ± 0.00^{b}

Data are presented as means ± Standard error. The means were obtained from three replicates (n=3). Different letters indicate significant differences between NM and M roots (for each nutrient) with increasing concentrations of $CaCO_3$ according to the LSD test ($p<0.05$). For calcium (Ca), no interaction was found between $CaCO_3$ concentrations and mycorrhizal colonization. Results were statistically compared separately: values with capital letters indicate significant differences between increasing $CaCO_3$ concentrations for NM and M roots separately

*Significant differences between NM and M roots according to the LSD test ($p<0.05$)

increase observed in non-mycorrhizal roots at 5 and 10 mM in comparison with roots grown on control medium (Table 2).

Impact of $CaCO_3$ on acid and alkaline phosphatase specific activities in chicory roots

In the medium without $CaCO_3$ (control), root acid phosphatase specific activity was significantly ($p<0.05$) higher (about 3 times) in *R. irregularis* mycorrhizal than in non-mycorrhizal roots. Whereas the root acid phosphatase specific activities were significantly ($p<0.05$) increased in non-mycorrhizal roots grown on $CaCO_3$ supplemented medium until reaching the maximum value of 1.5 nmol min^{-1} μg^{-1} of proteins, they remained constant in mycorrhizal roots (Fig. 7a).

Fig. 7 Root acid (**a**) and alkaline (b) phosphatase activity in transformed chicory roots (*C. intybus* L.) colonized or not by *R. irregularis* after 8 weeks of growth in the absence or in the presence of increasing $CaCO_3$ concentrations. Data are presented as means ± SE. Means were obtained from three replicates. Values with different letters indicate significant differences between increasing $CaCO_3$ concentrations. *Asterisks* indicate significant differences between mycorrhizal roots (*M*) and non-mycorrhizal roots (*NM*) according to the LSD test ($p<0.05$)

Concerning alkaline phosphatase activities, no significant differences were observed between mycorrhizal and non-mycorrhizal roots grown on media supplemented or not (control) with $CaCO_3$ (Fig. 7b). While increasing concentrations of $CaCO_3$ did not affect alkaline phosphatase activities in mycorrhizal roots, they were significantly decreased in non-mycorrhizal roots, except at 10 mM (Fig. 7b). These reductions were about 38% and 51% at 5 and 20 mM of $CaCO_3$, respectively.

Discussion

The pre-symbiotic (spore germination, germination hypha elongation) and the symbiotic (root colonization, extraradical hypha development, spore production) life cycle stages of the AM fungus *R. irregularis* were altered in vitro, but not completely inhibited, by increasing $CaCO_3$ concentrations (5, 10 and 20 mM). Thus, mycorrhizal colonization remained possible in the presence of high concentrations of $CaCO_3$ in the medium. A negative effect on AM formation has been reported previously for several other abiotic stresses like salinity (Ruiz-Lozano and Azcón 2000; Kaya et al. 2009), pollution (Debiane et al. 2009) and fungicides (Calonne et al. 2010), but no data are available concerning the impact of $CaCO_3$. Decreases in AM development could be due to the inhibition of spore germination (Hirrel 1981) and extraradical hypha growth (McMillen et al. 1998) as well as to the reduction of arbuscule numbers (Pfeiffer and Bloss 1988). The observed effects of $CaCO_3$ on the AM fungal development could be explained through the alteration of pH in the medium. In fact, in our experimental conditions, increases in the pH medium were recorded. In reality, $CaCO_3$ effects cannot be dissociated from a pH increase which is characteristic of alkaline substrates. A reduction in total arbuscular mycorrhizal root colonization and arbuscule formation by *Glomus intraradices* at high pH levels has been observed by van Aarle et al. (2002). Kumar et al. (2010) signalled that soil pH is one of the most important parameters that influence AM fungal sporulation, root colonization and extraradical mycelium formation. Consequently, inhibition of spore germination could induce adverse consequences on root colonization and AM fungal survival in soils.

Furthermore, as the main role of the mycorrhizal symbiosis is to improve the mineral nutrient uptake, the reduction in the mycorrhizal colonization, in particular, the arbuscular colonization (Ferrol et al. 2002), in the presence of $CaCO_3$ could have a negative impact on plant mineral nutrition and on plant growth. Our data showed that high $CaCO_3$ levels decreased chicory root growth. Surprisingly, this effect was more drastic in mycorrhizal roots than in non-mycorrhizal ones. This may be related to the allocation of a considerable portion of carbon by mycorrhizal roots to maintain the fungal metabolism (Eissenstat et al. 1993; Bryla and Eissenstat 2005). Inhibition of plant growth has also been observed in substrates having high concentrations of carbonates HCO_3^-(Valdez-Aguilar and Reed 2007) and NaCl (Kaya et al. 2009; Abed Alrahman et al. 2005).

Our findings showed that, in spite of the mycorrhizal root growth reduction under $CaCO_3$ stress, some mineral nutrient concentrations (P, N, Mg, Fe, Cu, Zn and Mn) were significantly higher in the mycorrhizal roots than in the non-mycorrhizal ones. The impact of $CaCO_3$ on mineral nutrient uptake in mycorrhizal roots is poorly documented. Cartmill et al. (2008) observed an increase in mineral absorption by mycorrhizal roots in the presence of high levels of carbonate. Al-Karaki (2006) noticed a better mineral absorption by tomato (*Solanum lycopersicum*) mycorrhizal plants in the presence of another abiotic stress, salinity.

Our results show that the AM symbiosis could enhance mineral nutrient uptake by chicory roots in the presence of high $CaCO_3$ concentrations. P is one of those minerals. An improvement in P absorption by mycorrhizal plants was observed by Cartmill et al. (2008) and Neumann and George (2004) under HCO_3^- high levels and water stress, respectively. Better assimilation of P represents one of the main mechanisms developed by plants to enhance their tolerance to abiotic stresses (Hirrel and Gerdemann 1980; Ojala et al. 1983), and for this reason a few authors have proposed mycorrhizal inoculation as a way to improve tolerance to high $CaCO_3$ levels in ectomycorrhizal and ericaceous plants (Clement et al. 1977; Lapeyerie and Chilvers 1985; Leake and Read 1989). Decreased absorption of P in alkaline soils is due to the precipitation of $H_2PO_4^-$ with Ca^{2+} to produce insoluble minerals not available to plants (Marschener 1995). This could explain the decrease in P concentrations in non-mycorrhizal chicory roots with increasing $CaCO_3$ levels in the medium substrate.

In the current study, the enhancement of mineral absorption by mycorrhizal roots in the presence of increasing $CaCO_3$ concentrations was observed even at low arbuscule frequency. Clark and Zeto (2000) attributed enhanced nutrient uptake to the greater root exploration of the substrate through the mycorrhizal hyphae. Activation and excretion of enzymes such as phosphatases by mycorrhizal roots or/and the AM fungal hyphae (Marschner 1998) that increase the availability and translocation of minerals (Al-Karaki 2006) could also explain the enhanced mineral uptake. In our experimental conditions, we showed that whereas acid and alkaline phosphatase activities remained constant in mycorrhizal roots, these enzymatic activities were disturbed in non-mycorrhizal roots grown in the presence of $CaCO_3$ when compared to the control.

However, no clear correlation between phosphatase activities and enhanced P absorption could be established, in contrast mycorrhizal Madagascar Periwinkle (*Catharanthus roseus*) (Cartmill et al. 2008) and carrots (*Daucus carota*) roots (Koide and Kabir 2000), where better P nutrition was attributed to the improvement of root alkaline phosphatase activities. It would be interesting to investigate whether the higher P concentrations in the in vitro mycorrhizal chicory roots, compared to non-mycorrhizal ones, could be explained by the activity of AM fungal P transporters (Harrison and van Buuren 1995; Maldonado-Mendoza et al. 2001).

In conclusion, the presence of $CaCO_3$ in monoxenic conditions impaired *R. irregularis* spore germination and symbiosis development in chicory roots without inhibiting the AM fungal life cycle completely. Not only AM symbiosis establishment remained possible, but it also proved to be beneficial to mineral nutrient uptake by the plant roots. However, phosphatase activities appear to be of little importance in P uptake by mycorrhizal roots under a $CaCO_3$ stress.

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