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Calcium Uptake by Alfalfa as Modified by a Mycorrhizal Fungus and Liming

M. BERMUDEZ[1] and R. AZCON[2*]
[1]*Centro de Investigaciones Agronómicas, Universidad de Costa Rica, San Jose, Costa Rica; and* [2]*Departamento de Microbiología, Estación Experimental del Zaidín (CSIC), Prof. Albareda 1, Granada 18008, Spain. Tel. +34-958-121011, Fax. +34-958-129600*

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Abstract

The purpose of this experiment was to study the effect of an arbuscular mycorrhizal (AM) fungus on calcium acquisition by *Medicago sativa* plants grown in a medium at five levels of $CaCO_3$. Determinations of dry matter production and concentrations of N, P, K, Ca and Mg in plant tissues showed that mycorrhizal colonization increased content of N, P and K and overall yield, but decreased the concentration of Ca and Mg in plant shoots. The response of mycorrhizal colonization to different amounts of extractable soil Ca indicated that, while mycorrhiza formation was not sensitive to soil Ca it induced a reduction of Ca in the plant. This study demonstrated that AM fungi modulate the ion ratios and ion balance in the plant. In general, the nutrient/Ca ratios were higher in AM than in non-AM plants. These results show that AM formation enhanced N, P and K and decreased Ca uptake. The mycorrhizal condition affected plant tolerance to nutrient deficiency or excess by modifying uptake. Only in AM plants were differences observed between the effects of varying Ca availability. There was a positive correlation between plant Ca content and nodulation in the mycorrhizal treatments. On the other hand, nodulation was not correlated with N, P and K contents. Calcium amendments had no effect on nodule formation in non-mycorrhizal plants, but nodule production was highly increased in AM plants, particularly at the highest Ca level. The correlation coefficients for parameters

*The author to whom correspondence should be sent.

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related to absorption of nutrients in mycorrhizal plants were highly significant for N and P, as well as for Ca and nodulation. According to our results, the mycorrhizal effect on Ca assimilation was not a simple buffering effect associated with Ca excess. The impact of AM function is dependent on various soil and environmental factors. The mechanisms by which AM fungi are able to alleviate nutrient excess has yet to be understood. Further research will be necessary to elucidate this effect.

Keywords: Arbuscular mycorrhiza, Ca uptake, legume symbiosis

1. Introduction

Soil fertility is associated with mineral deficiencies and/or toxicities which affect plant nutrition. Arbuscular mycorrhizal (AM) fungi are important soil microorganisms which affect processes in the soil-plant system and nutrient availability to plants (Barea et al., 1993). The AM symbiosis can modulate plant behaviour in response to changes in soil nutrient content. AM fungi are important not only for plant growth but also for plant establishment and plant competition in low fertility soils (Barea, 1991). There are few studies on the capacity of external mycorrhizal hyphae for nutrient uptake and delivery (with the exception of studies concerning P). Further quantification will be required in order to properly test the role of AM fungi in the acquisition of mineral sources by a host plant according to environmental conditions.

Mycorrhizal plants accumulate less Mn (Arines et al., 1989), Mg and Ca (Azcón and Barea, 1992) and are more tolerant of metal toxicity, in spite of the benefit derived from AM association in the uptake of nutrients with low mobility in the soil (Harley and Smith, 1993; Smith and Gianinazzi-Pearson, 1988). Arbuscular mycorrhizae improve plant growth under a variety of environmental conditions, including stress. They can also mitigate nutritional imbalances (Hale and Orcutt, 1987). The mechanisms involved in such an effect have not yet been well established.

In a previous experiment (Azcón and Barea, 1992) the legume *Medicago sativa* L. was grown in four calcareous soils which had high concentrations of extractable Ca. Data regarding AM effects on dry matter and nutrient content in plant tissues demonstrated that N, P and K (but not Ca and Mg) increased in the host plant via the mycorrhizal fungus. Based on these observations the authors concluded that the AM endophyte depressed excessive Ca acquisition. There is little published information concerning the role of AM fungi in the buffering effect in the presence of nutrient excess. Observations by our group on Ca uptake by AM plants required specifically-designed experiments to test the effect of mycorrhizal fungi on a range of Ca levels in the rooting medium. It is

important to establish the role that mycorrhizal symbiosis plays in response to limited, optimal or supraoptimal availability of soil Ca.

The objective was to study the ability of mycorrhizal alfalfa (*Medicago sativa* L.) to regulate plant nutrition against a background of increasing levels of soil Ca. As previous work with this plant has suggested, the mycorrhizal effect associated with excess nutrient availability (Azcón and Barea, 1992) in determining host-plant responses is not clear. This study aims to determine the effects of different levels of calcium fertilization on nutrient uptake by nodulated alfalfa infected by an AM fungus.

2. Materials and Methods

Alfalfa (*Medicago sativa* L cv. Aragon) was the test plant. At planting all pots received 2 ml of standard inoculum of *Rhizobium meliloti*. The inoculum of this bacterium was grown in Allen (1957) medium and applied (2 ml containing 10^8/cell) at sowing into both mycorrhizal and non-mycorrhizal pots. Alfalfa plants were grown for 10 wk in 500 ml pots filled with a sand/vermiculite/soil (2:2:1, v:v:v) medium, (100 g/pot). The substrate mixture was steam-sterilized (100°C, 1 h, on 3 consecutive days). The test soil was collected from the Alpujarras area in Granada Province, Spain. Soil characteristics were: 0.47% organic matter; 0.1 mg P/kg soil extracted with 0.5M, $NaHCO_3$ (Olsen P); 1 g/kg of total N, 5.25 g/kg K, 40 g/kg equivalent Ca and 3.9 g/kg active Ca.

The sterile soil/vermiculite/sand mixture was divided into six batches. Untreated control and five levels of $CaCO_3$ (g/kg soil) were added to the potting medium as follows: 0, 16, 24, 60, 80, and 100. After the lime applications the pH was determined in the resulting medium. In the three treatments selected (0, 60, and 100 g Ca CO_3/kg) the results were: pH 6.9 (in the control); pH 7.6 (60 g/kg) and pH 7.9 after 100 g/kg $CaCO_3$ application. Half of the pots were noninoculated and the rest were inoculated with the AM fungus *Glomus fasciculatum* (Taxter sensu Gerd) Gerd and Trappe. Inoculation was carried out using a stock culture of the fungus stored for 6 to 9 months in a polyethylene box at 5°C. The inoculum consisted of thoroughly mixed rhizosphere samples containing 4 spores per g, mycelium, and highly colonized mycorrhiza root fragments (85%). Inocula (3 g/pot) were applied directly to the planting hole at sowing time. Soil extract [2 ml/pot of soil/water (equal v/v) filtered through Watman N° 1 paper] was added to reintroduce the native microbial population except for AM propagules.

Plants were grown in a controlled chamber at 20 to 22°C during the day and at 12 to 17°C at night. The photoperiod was 16/8 h light/dark and the relative humidity was 70 to 90%. The photosynthetic photon flux density was 503

mmol/m^2/s as measured with a lightmeter (LICOR, model LI-188B). During the assay each pot was treated weekly (75 ml/pot) with a nutrient solution described by Hepper and O'Shea (1984) lacking Ca and modified to contain N and P at 1/2 strength. In a previous assay we found that this nutrient regime permitted a high level of AM-fungal development without limiting plant growth.

Plants were harvested after a growth period of 10 wk. Shoot and root dry weights were recorded. Plant leaves were weighed and dried at 70°C for 1 d and ground in a Willey Mill (0.5 mm mesh). Materials were digested with a 1:1 nitric:perchloric acid mixture. Concentrations of N and P were colorimetrically measured on a Technicon auto-analyzer (Anon., 1974). Concentrations of K were determined by flame photometry and that of Ca and Mg by atomic absorption spectrophotometry using a Perkin-Elmer 5000 spectrophotometer.

After staining by the procedure of Phillips and Hayman (1970), AM infection was assessed microscopically using the gridline-intersect method of Giovannetti and Mosse (1980). The nodules on the main and lateral fresh roots were counted with a magnifying glass.

Data were subjected to a two-way analysis of variance with randomized complete blocks with the following factors: mycorrhizal inoculation (2 levels) and level of fertilization (6 levels). When the main effect was significant ($P<0.05$), differences between means were evaluated for significance by using Duncan's multiple-range test in an orthogonal design.

3. Results

Alfalfa shoot and root dry matter were not influenced by the amount of Ca added to the growth medium. Plant growth was increased by mycorrhizal colonization (Table 1). Ca amendments increased the number of nodules only in mycorrhizal plants and particularly when Ca was added at the three highest concentrations. Nodule formation increased only slightly under the three lowest Ca regimes but was deeply affected by the three highest Ca regimes in response to mycorrhizal colonization. Mycorrhiza development was not affected by the calcium treatments (Table 1).

Shoot P concentration (Table 2) was stimulated by mycorrhizal colonization. Increasing amounts of calcium did not significantly affect plant N, P or K concentration. Similar trends were found regarding N, P and K contents (Table 3). Nutrient uptake of N and P, but not of K, was stimulated by the AM fungus and was most effective with 60 g $CaCO_3$/kg. However, the AM effect on the Ca and Mg concentrations in alfalfa shoots (Table 4) was one of significant

decrease. Mycorrhizal plants accumulated more Ca under high than under low Ca regimes.

Table 1. Dry matter (mg) and formation of microbe root symbioses of non-mycorrhizal (–) and mycorrhizal (M) alfalfa plants supplied with increasing levels of $CaCO_3$.

$CaCO_3$ (g/kg)	Dry weight Shoot		Root		Number of Nodules		Root length mycorrhizal (%)	
	–	M	–	M	–	M	–	M
–	311a	478b	324a	629b	2a	5b	–	47a
16	305a	544bc	347a	689b	3a	8b	–	30a
24	275a	579c	334a	609b	3a	6b	–	34a
60	329a	626c	270a	589b	2a	30c	–	34a
80	322a	539bc	260a	680b	3a	20c	–	43a
100	385ab	628c	341a	712b	2a	20c	–	31a

For each parameter (pair of columns) values followed by the same letters are not significantly different ($P<0.05$) using Duncan's multiple range test (n = 5).

Table 2. Percentage of N, P and K in the shoots of non-mycorrhizal (–) and mycorrhizal (M) alfalfa shoots supplied with increasing levels of $CaCO_3$.

$CaCO_3$ (g/kg)	N (%)		P (%)		K (%)	
	–	M	–	M	–	M
–	3.20a	4.15b	0.11a	0.16c	2.75a	2.76a
16	3.20ab	3.38ab	0.11a	0.14cb	2.51a	2.50a
24	3.37a	3.34a	0.10a	0.14cb	2.71a	2.54a
60	3.50ab	3.90ab	0.11a	0.16c	2.99a	2.53a
80	3.71ab	3.26a	0.11a	0.14cb	2.61a	3.02a
100	3.43ab	3.31ab	0.12b	0.12ab	2.79a	2.89a

For each parameter (pair of columns) values followed by the same letters are not significantly different ($P<0.05$) using Duncan's multiple range test (n = 5).

Table 3. N, P and K content in the shoots of non-mycorrhizal (–) and mycorrhizal (M) alfalfa shoots supplied with increasing levels of $CaCO_3$.

$CaCO_3$ (g/kg)	N (mg)		P (mg)		K (mg)	
	–	M	–	M	–	M
–	9.9a	19.7c	0.35a	0.75b	8.5a	13.6c
16	9.9ab	18.3c	0.35a	0.76b	7.9a	13.1c
24	10.3ab	19.1c	0.36a	0.79bd	8.3a	15.3c
60	12.0ab	24.2d	0.37a	0.97d	10.2b	15.9c
80	12.3b	17.6c	0.35a	0.74b	8.6ab	16.4c
100	12.6b	20.5c	0.43a	0.74b	10.3ab	18.3c

For each parameter (pair of columns) values followed by the same letters are not significantly different ($P<0.05$) using Duncan's multiple range test ($n = 5$).

Table 4. Ca and Mg (concentration and content) in non-mycorrhizal (–) and mycorrhizal (M) alfalfa shoots supplied with increasing levels of $CaCO_3$.

$CaCO_3$ (g/kg)	Ca (%)		Ca (mg)		Mg (%)		Mg (mg)	
	–	M	–	M	–	M	–	M
–	3.44c	1.88b	10.8b	8.9a	0.98b	0.55a	3.0b	2.5a
16	4.42d	1.78a	14.0cb	9.7a	1.11c	0.59a	3.5b	3.2b
24	4.16d	1.64a	12.8cb	9.7a	0.88b	0.53a	2.7a	3.0b
60	3.53c	2.39b	12.4cb	14.7c	0.91b	0.53a	3.1ab	3.3b
80	3.60c	2.37b	11.9cb	12.8c	0.97b	0.52a	3.2b	2.8a
100	3.34c	2.34ab	13.2cb	14.7c	0.96b	0.51a	3.7b	3.2a

For each parameter (pair of columns) values followed by the same letters are not significantly different ($P<0.05$) using Duncan's multiple range test ($n = 5$).

Mycorrhization improved the accumulation of Ca in plant shoots only under the three highest levels of Ca in the medium.

Nutrient/Ca ratios (Table 5) show differences between AM and non-AM plants irrespective of the calcium treatments. For all nutrients the mycorrhizal symbiosis had a role in increasing these ratios. The extent of such improvement for all nutrients was highest with the lowest Ca supply. The linear correlation

coefficients for parameters related to absortion of nutrients in mycorrhizal plants (Table 6) were highly significant for N and P as well as for Ca and nodulation.

Table 5. Nutrients/Ca ratio in the shoots of non-mycorrhizal and mycorrhizal alfalfa shoots supplied with increasing levels of $CaCO_3$.

$CaCO_3$ (g/kg)	N/Ca		P/Ca		K/Ca		Mg/Ca	
	–	M	–	M	–	M	–	M
–	0.93a	2.21c	0.030a	0.085c	0.79a	1.46c	0.28b	0.29bc
16	0.72a	1.90bc	0.025a	0.078c	0.57a	1.40c	0.25b	0.33c
24	0.81a	2.04c	0.024a	0.085c	0.65a	1.55c	0.21a	0.32c
60	0.99a	1.63b	0.031a	0.067b	0.84ab	1.06b	0.26b	0.22a
80	1.03a	1.37b	0.030a	0.059b	0.72a	1.27b	0.27b	0.22a
100	1.00a	1.41b	0.032a	0.051b	0.81a	1.23b	0.28b	0.22a

For each parameter (pair of columns) values followed by the same letters are not significantly different ($P<0.05$) using Duncan's multiple range test ($n = 5$).

Table 6. Lineal correlation coefficients for parameters related to absortion of nutrients for different calcium additions in mycorrhizal alfalfa shoots.

	N	P	K	Ca
P	0.766**	–		
K	0.394*	0.418*	–	
Ca	0.388*	0.147	0.456*	–
Nod	0.342	0.271	0.078	0.677**

*$P \leq 0.05$; **$P \leq 0.001$

4. Discussion

In a previous study, Azcón and Barea (1992) reported on the buffering effect of the AM fungus *G. mosseae* in the presence of high Ca concentrations. In the

present study the same effect has been confirmed for plants colonized by *Glomus fasciculatum*.

By supplying the radioisotope (^{45}Ca) it has been shown that the external hyphae of AM have a capacity for Ca uptake and subsequent transport to the host root (Rhodes and Gerdemann, 1978). There is no conclusive direct experimental evidence for Mg uptake and transport in mycorrhizal hyphae (Kothari et al., 1990).

The nutrient/Ca ratios in mycorrhizal plants indicate that increases in N, P and K are relative to Ca. Major ions can influence each others ion absorption rates in the cell by interacting competitively or by affecting ion selectivity in membranes (Epstein, 1972; Grattan and Grieves, 1993; Liu et al., 1994; Tomlinson, 1990). The major quantitative functions of Ca are extracellular (Wyn Jones et al., 1979). Ca in xylem sap is a determinant of stomatal behaviour and other physiological events (Atkinson, 1991). Ca affects membrane stabilization, integrity, permeability and transport all of which affect cell metabolism and are of particular relevance for nutrient exchanges through plasmalemmas, as can be seem in root-fungal symbiotic association (Smith and Gianinazzi-Pearson, 1988). While percentages of AM colonization did not change with the Ca regimes, the effect of AM activity on nutrition in above-ground biomass was relative to Ca levels. In the present, as in a previous study nodulation was stimulated by mycorrhiza (Azcón and Barea, 1992). This effect was particularly evident at the highest amounts of Ca in the medium. Mycorrhizal mechanisms which altered nutrient/Ca ratios may be involved in the effect on nodulation.

In the present study the soil pH changes caused by Ca applications affected neither mycorrhizal colonization nor plant growth in non-AM treatments. These modifications in the pH do not seem to play any significant part in the parameters evaluated.

The differential nutrient uptake and/or translocation to the shoots might enable mycorrhizal plants to colonize the most adverse environments (Lambais and Cardoso, 1988; Poovaiah, 1993).

Further studies will be required in order to understand the putative role of Ca^{2+} in mycorrhizal plant protection mechanisms against physical or chemical environmental factors. The mechanism by which AM fungi control Ca uptake and translocation are not yet known. Experiments are presently being carried out to clarify these aspects of mycorrhizal behaviour.

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