Biol Fertil Soils (1997) 24:81–86

ORIGINAL PAPER

R. Azcón · F. El-Atrash

Influence of arbuscular mycorrhizae and phosphorus fertilization on growth, nodulation and N_2 fixation (^{15}N) in *Medicago sativa* at four salinity levels

Received: 5 September 1995

Abstract The role of an isolate of the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* in the protection of *Medicago sativa* (+*Rhizobium meliloti*) against salt stress induced by the addition of increasing levels of soluble salts was studied. The interactions between soluble P in soil (four levels), mycorrhizal inoculum and degree of salinity in relation to plant growth, nutrition and infective parameters were evaluated. Salt stress was induced by sequential irrigation with saline water having four concentrations of three salts (NaCl, $CaCl_2$, and $MgCl_2$). ^{15}N-labelled ammonium sulphate was added to provide a quantitative estimate of N_2 fixation under moderate to high salinity levels. N and P concentration and nodule formation increased with the amount of plant-available P or mycorrhizal inoculum in the soil and generally declined as the salinity in the solution culture increased from a moderate to a high level. The mycorrhizal inoculation protected the plants from salt stress more efficiently than any amount of plant-available P in soil, particularly at the highest salinity level applied (43.5 dS m^{-1}). Mycorrhizal inoculation matched the effect on dry matter and nutrition of the addition in the soil of 150 mg P kg^{-1}. Nevertheless the highest saline solution assayed (43.5 dS m^{-1}) affected more severely plants supplemented with phosphorus than those with the addition of mycorrhizal inoculum. Such a saline-depressing effect was 1.5 (biomass), 1.4 (N) and 1.5 (P) times higher in plants supplied with soluble phosphate than with AM inoculum. Mechanisms beyond those mediated by P must be involved in the AM-protective effect against salinity. The ^{15}N methodology used allowed the determination of N_2 fixation as influenced by different P applications compared to mycorrhizal inoculation. A lack of correlation between nodule formation and function (N_2 fixation) was evidenced in mycorrhizal-inoculated plants. In spite of the reduced activity per nodule in mycorrhizal-inoculated plants, the N contents determined indicated the highest acquisition of N occurred in plants with the symbiotic status. Moreover, N and P uptake increased while Ca and Mg decreased in AM-inoculated plants. Thus P/Ca ratios and cation/anion balance in general were altered in mycorrhizal treatments. This study therefore confirms previous findings that AM-colonized plants have optional and alternative mechanisms available to satisfy their nutritive requirements and to maintain their physiological status in stress situations and in disturbed ecosystems.

Key words Arbuscular mycorrhiza · Salt tolerance · N_2 fixation · ^{15}N-labelled fertilizers · *Glomus mosseae* · *Medicago sativa* · *Rhizobium meliloti* · Soluble P

R. Azcón (✉) · F. El-Atrash
Department of Microbiology, Estación Esperimental del Zaidin, CSIC, Prof. Albareda 1, E-18008-Granada, Spain

Introduction

Arid saline soils inhibit or reduce plant survival and development. The major causes of saline toxicity in plants include an unfavourable pH, an imbalance of essential cations and anions and an altered water-holding capacity. Often in arid or semiarid zones (approximately 7% of the Earth's land surface) crop production is low. The influence of soil salinity on the nutrition of plants is not well understood, but nutrient uptake is known to be affected by the osmotic potential of the soil solution. The symptoms of salinity stress resemble those of P deficiency (Maas and Nieman 1978). Plants relying on a symbiotic relationship for adequate mineral nutrition and water uptake may differ in salt tolerance depending on the tolerance to soil salinity of the plant and symbiont (Reddell et al. 1986).

Arbuscular-mycorrhizal (AM) fungi occur naturally in most soils and may improve the growth of many plants under a variety of stress situations (Allen and Boosalis 1983). The mechanism of salt tolerance may involve improved P nutrition since the application of P has been clearly shown to improve yield under saline conditions (Hirrel and Gerdemann 1978; Champagnol 1979). Preliminary investigations suggest rhizosphere organisms may be influenced by salt

accumulation in soil (Barrett-Lennard et al. 1986). The effects of salinity on symbiotic relationships (mycorrhizal and legume *Rhizobium* symbiosis) have hardly been assessed. Indeed plant salt tolerance varies with the rhizosphere symbionts (Dixon et al. 1993a). Salt may be important in the survival, reproduction and spread of mycorrhizal fungi and *Rhizobium* in saline soils. Mycorrhiza, phosphate, water and ion balance are ecosystem components that strongly affect biological N_2 fixation by legume-*Rhizobium* symbiosis (Azcón et al. 1988; Sprent 1986). In previous studies species and isolates of AM fungi have demonstrated large variations in salt tolerance (Rosendahl and Rosendahl 1991).

The objective of our research was to compare the effect of *Glomus mosseae* with three P applications on yield, nutrition and N_2 fixation (^{15}N) in alfalfa subjected to increasing levels of salinity induced by the application of four concentrations of three salts (NaCl, $CaCl_2$ and $MgCl_2$). ^{15}N methodology was used to determine N_2 fixation in the different P-amended soils compared to mycorrhizal inoculation at all salinity levels. To avoid the indirect influence of the mycorrhizal fungus on improved P nutrition, we compared the stress tolerance of mycorrhizal-inoculated plants to non-mycorrhizal-inoculated P-supplemented plants of a similar size.

Materials and methods

Experimental design

The experiment was carried out with the following treatments: non-mycorrhizal control, application of three increasing amounts of a soluble phosphate fertilizer and *G. mosseae* as a mycorrhizal inoculum at four salinity levels. These 25 treatments were replicated 5 times, giving a total of 125 pots in a randomized block design.

Plant, soil and pot experiments

Alfalfa, *Medicago sativa* L. cv. Aragon, was used as the test plant. Five-day-old seedlings were transplanted into pots filled with a steam-sterilized (three consecutive 1-h sterilizations) 5:2 (v/v) soil/sand mixture. Sieved (2 mm) soil with the following characteristics was used: 28.0% clay, 49.0% silt, 22.5% sand, 1.2% organic matter, pH 7.5, 11.0 mg P kg^{-1} (Na-HCO_3-extractable P), 0.63 meq K 100 g^{-1}, and 0.1% N total, 41.5 meq Ca 100 g^{-1} and 4.9 meq Mg 100 g^{-1}. Electrical conductivity was 2.5 dS m^{-1}.

The experimental soil was divided into five batches: C (untreated control), P_{50}, P_{100} and P_{150} (dose equivalents of H_2KPO_4 added in mg kg^{-1} soil) and M (mycorrhizal inoculum of *G. mosseae*). After incubation at 19°–25°C, with suitable watering, for 2 weeks, the soil was analysed for plant-available P (Olsen). The results (in mg P kg^{-1} soil) after incubation and just before planting were: 11, 16, 21 and 27 mg P kg^{-1}soil. Five-day-old seedlings were transplanted (three plants per pot) into pots containing 500 g experimental soil thoroughly mixed and incubated with the corresponding amount of soluble phosphate or the mycorrhizal inoculum.

Microbial treatments

The mycorrhizal inoculum from a stock culture of *G. mosseae* isolate (Nicol.&Gerd.), Gerd.&Trappe, was applied at 5 g pot^{-1} consisting of soil with spores, mycelia and infected root fragments. Five millilitres of inoculum filtrate containing the microbial population except for propagules of mycorrhizae was obtained by suspending 100 g mycorrhizal inoculum from *G. mosseae* in 600 ml sterile water. The suspension was filtered (Whatman No. 1) after shaking and decanting. A standard inoculum of *Rhizobium meliloti* (1 ml 10^7 cfu ml^{-1}) was applied to all pots at transplanting.

Environmental growth conditions

The plants were grown in the greenhouse under controlled environmental conditions of a 16/8 h light/dark cycle, a 25°/15°C day/night temperature, 50% relative humidity and with a photosynthetic photon flux density (PPFD) of 500–750 µmol m^{-2} s^{-1}. Water was supplied after daily weighing to maintain the water-holding capacity (WHC) of the test soil/sand mixture at near 100% throughout the experiment. The plants were grown for 20 days before being exposed to four levels of increasing salt concentrations of sodium chloride (NaCl), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$). The four levels of saline irrigation water (13.8; 22.2; 28.8 and 43.5 dS m^{-1}) were produced by the appropriate dilution of a mixture of NaCl, $CaCl_2$ and $MgCl_2$ in the proportion 3/2/1, achieved by adding 0.360 g l^{-1} NaCl/0.252 g l^{-1} $CaCl_2$/0.117 g l^{-1} $MgCl_2$ in four increasing concentrations. Plants were exposed to salinity by successive additions (20 ml pot^{-1}) supplied weekly for 9 weeks. A total amount of 180 ml of the corresponding saline solution was added per pot along the experiment. In a parallel assay containing control pots without plants under similar experimental conditions, one of the four levels of saline solution was applied at the same time to give at the end of the experiment the following soil conductivity rates: 9.2, 14.8, 19.2 and 29 dS m^{-1}. Each saline dilution was applied to five replicate pots in each treatment. After 2 weeks from transplanting and before applying the saline solution, each pot was fertilized with a solution of $(^{15}NH_4)_2$ SO_4 with 10% ^{15}N. A dose equivalent to 5 kg N ha^{-1} (1 mg N 500 g^{-1} soil) was applied.

Determination of growth and symbiotic parameters

At harvest, shoot dry weight was recorded after drying at 70°C. Concentrations of N and P were colorimetrically measured on a Technicon autoanalyser (Anon 1974). Ca and Mg were determined by atomic absorption spectrophotometry using a Perkin-Elmer 5000 spectrophotometer. AM colonization was microscopically assessed using the gridline intersect method of Giovannetti and Mosse (1980) after staining by the procedure of Phillips and Hayman (1970). The roots were carefully washed and the number of nodules was assessed visually. The N isotopic composition of plant shoots was determined by mass spectrometry as described by Fiedler and Proksch (1975).

Statistical analysis

Data were subjected to a randomized block analysis of variance. Treatments were differentiated with Duncan's multiple range test by the least significant difference method (LSD, $P<0.05$).

Results

Dry weights of alfalfa were increased by P fertilization or mycorrhizal colonization at the different levels of salinity (Table 1). Plants under high salinity grew better with mycorrhizal colonization than those with P fertilization. Indeed the effect of mycorrhizal colonization was similar to that of P fertilization at the two lowest levels of salinity (2.5 and 13.8 dS m^{-1}), but at increasing conductivities (22.2, 28.8 and 43.5 dS m^{-1}) plants inoculated with *G. mosseae* showed better growth than plants fertilized with P. The

Table 1 Effect of different salinity levels on mean shoot dry weight (mg pot^{-1}) of non-inoculated control (C), P-fertilized (P_{50}, P_{100} and P_{150}) or mycorrhizal-inoculated (M) alfalfa plants

Saline solution (dS m^{-1})	Treatment				
	C	P_{50}	P_{100}	P_{150}	M
0	1040gf	1470h	1460h	1680h	1520h
13.8	940f	970f	1060fg	1120g	1090g
22.2	720c	760c	780c	840d	910e
28.8	570a	610ab	650b	640b	770c
43.5	450a	490ab	550b	450a	680c

Means (of five replicates) not followed by a common letter differ significantly ($P<0.05$)

Table 3 Nodule number (Nno), percentage of mycorrhizal root length (%AM) and atom percent ^{15}N excess in non-mycorrhizal-inoculated (C) P-fertilized (P_{50}, P_{100}, and P_{150}) or mycorrhizal-inoculated (M) alfalfa plants according to salinity level

Saline solution (dS m^{-1})	Treatment					
	C Nno	P_{50} Nno	P_{100} Nno	P_{150} Nno	M	
					Nno	% AM
0	88.0g	93.6gh	110.6gh	121.1h	153i	40b
13.8	79.2f	107.2h	111.6h	158.2i	160i	51c
22.2	58.6f	60.4f	65.8f	111.2gh	153i	51c
28.8	12.8b	18.6d	5.8f	8.8b	33e	37b
43.5	1.2	4.0ab	6.6b	9.0b	16d	19a
	^{15}N (%)	^{15}N (%)	^{15}N (%)	^{15}N (%)	^{15}N (%)	
0	0.050c	0.045b	0.035a	0.035a	0.044b	
13.8	0.076d	0.069d	0.076d	0.070d	0.078d	
22.2	0.096e	0.102ef	0.101e	0.110ef	0.106f	
28.8	0.129h	0.129h	0.116fg	0.142i	0.113g	
43.5	0.151li	0.136hi	0.130h	0.149i	0.117g	

Means (of five replicates) not followed by a common letter differ significantly ($P<0.05$)

yield of mycorrhizal-inoculated plants under 43.5 dS m^{-1} was similar to that of controls or plants in the P_{50} and P_{100} treatments grown at 22.2 dS m^{-1}. Increased salinity decreased plant biomass; this detrimental effect was higher in P-amended plants than in plants in the control of mycorrhizal-inoculated treatments. Moreover, P fertilization had no effect on growth response at the highest salinity level. The effect of the P supplement on plant growth and nutrient uptake diminished as the salinity increased in the medium.

N and P acquisition by plants (Table 2) was negatively influenced by the salinity in the medium. Nevertheless, the N and P contents in mycorrhizal plants were reduced: 3.6 (N) and 2.8 (P) times under the high level of salinity, whereas in P_{150}-fertilized plants such a detrimental effect was greater, the uptake of these both nutrients being reduced 4.6-fold (N) and 4.5-fold (P).

Nodules formed in mycorrhizal root systems were generally more abundant than in the P-fertilized plants (Table 3). The two highest levels of salinity significantly reduced the number of nodules formed in all the treatments. This negative effect of salinity on nodulation was moderated by mycorrhizal colonization at 22.2, 28.8 and 43.5 dS m^{-1}. The ^{15}N isotopic composition of alfalfa shoots was greatly altered by the salt content in all the treatments (Table 3). ^{15}N enrichment of plant shoots increased with salinity, but diminished in mycorrhizal-inoculated plants compared with control of P-amended plants subjected to the highest salinity level (43.5 dS m^{-1}).

A high salinity also affected mycorrhizal colonization by *G. mosseae*, but to a lesser degree than nodulation (Table 3). Ca and Mg concentrations in shoots of alfalfa plants were decreased by mycorrhizal colonization (Table 4). Calcium accumulation was diminished in mycorrhizal-

Table 2 Shoot N and P concentration and content (on a per pot basis) of non-inoculated control (C), P-fertilized (P_{50}, P_{100} and P_{150}) or mycorrhizal-inoculated (M) alfalfa plants according to salinity level

Saline solution (dS m^{-1})	Treatment									
	C		P_{50}		P_{100}		P_{150}		M	
	%N	N (mg)	%N	N (mg)	%N	N (mg)	%N	N (mg)	%N	N (mg)
0	2.3ab	24.3d	2.6b	38.5f	3.1c	46.0g	3.5d	59.0h	4.4f	67.8h
13.8	2.9b	26.9d	2.8bc	27.2d	3.2cb	33.9f	3.3c	37.3f	4.4f	48.0g
22.2	4.6f	32.9d	2.7b	20.5c	2.5ab	19.5c	2.4ab	20.4c	3.1cb	28.1d
28.8	3.8d	18.9b	3.8d	23.3c	2.5ab	16.6b	2.8b	18.0b	2.6b	20.0c
43.5	2.1a	9.2a	2.5ab	12.8ab	3.0b	16.4b	2.8ab	12.8b	2.7ab	18.5c
	%P	P (mg)	%P	P (mg)	%P	P (mg)	%P	P (mg)	%P	P (mg)
–	0.11b	1.2c	0.12bc	1.8f	0.14c	2.0f	0.15c	2.5g	0.17d	2.6g
13.8	0.13bc	1.3d	0.15c	1.4d	0.18d	1.0f	0.17d	2.0f	0.22f	2.4g
22.2	0.17d	1.3d	0.13b	1.0c	0.12b	0.9c	0.13b	1.1c	0.15c	1.4d
28.8	0.09a	0.5b	0.12bc	0.7c	0.12bc	0.8c	0.12b	0.8c	0.15c	1.2d
43.5	0.08a	0.4a	0.11b	0.5b	0.11abc	0.6b	0.12bc	0.6b	0.13bc	0.9d

Means (of five replicates) not followed by a common letter differ significantly ($P<0.05$)

Table 4 Shoot Ca and Mg concentration (on a per pot basis) and P/Ca ratio of non-inoculated control (C), P-fertilized (P_{50}, P_{100} and P_{150}) or mycorrhizal-inoculated (M) alfalfa plants according to salinity level

Saline solution (dS m^{-1})	Treatment									
	C		P_{50}		P_{100}		P_{150}		M	
	%Ca	%Mg	%Ca	%Mg	%Ca	%Mg	%Ca	%Mg	%Ca	%Mg
–	4.4 d	0.62 c	4.2 d	0.62 c	3.7 cd	0.55 bc	3.9 d	0.58 c	2.5 b	0.43 ab
13.8	3.8 cd	0.54 bc	3.2 c	0.51 c	3.3 c	0.51 b	2.9 c	0.46 b	2.1 a	0.37 a
22.2	3.1 c	0.50 b	3.4 c	0.51 bc	3.5 c	0.53 bc	3.1 c	0.52 bc	2.4 ab	0.40 ab
28.8	3.2 c	0.56 bc	3.6 cd	0.55 c	3.4 c	0.56 bc	3.6 c	0.58 c	3.1 c	0.47 b
43.5	3.7 cd	0.61 bc	3.9 cd	0.65 bc	4.2 d	0.60 bc	4.3 d	0.41 ab	4.2 d	0.40 ab
	P/Ca		P/Ca		P/Ca		P/Ca		P/Ca	
–	0.025		0.028		0.038		0.038		0.068	
13.8	0.034		0.047		0.054		0.059		0.105	
22.2	0.055		0.038		0.034		0.042		0.062	
28.8	0.028		0.033		0.035		0.033		0.048	
43.5	0.022		0.028		0.026		0.028		0.031	

Means (of five replicates) not followed by a common letter differ significantly ($P<0.05$)

inoculated plants particularly under the less severe salinity levels. There were large differences in P/Ca ratios between mycorrhizal-inoculated and non-mycorrhizal-inoculated plants irrespective of salinity or phosphate treatment (Table 4).

Discussion

The results from this study agree with previous data (Azcón et al. 1988; Rosendahl and Rosendahl 1991; Pfeiffer and Bloss 1988; Dixon et al. 1993b) showing that mycorrhizal symbiosis increases the growth and nutrition of plants under saline conditions. Plant growth and nutrient uptake diminished as salinity in the medium increased. The application of P clearly improved yield and nutrition under saline conditions. The mechanism of salt tolerance may involve improved P nutrition since non-mycorrhizal-inoculated plants with P fertilization grew as well as mycorrhizal-inoculated plants without P addition. The mechanism of mycorrhizal effects found under light salinity levels (13.8 dS m^{-1}) seems to be in large part due to a indirect P-mediated effect. Nevertheless at the highest levels of salinity applied the detrimental effect on growth and nutrition of salt was less severe in mycorrhizal-colonized plants than reported previously (Ojala et al. 1983). The P concentrations in the tissues of mycorrhizal-inoculated and P_{150}-fertilized plants were similar (0.12 and 0.13% P, respectively) under the highest salinity level applied. Nevertheless there was a significant drop in plant biomass of P-fertilized plants compared to that of mycorrhizal-inoculated plants. The detrimental saline effect could not be only correlated to host P nutrition. These findings allow us to deduce that the increased salinity tolerance in mycorrhizal plants is based on additional effects other than P nutrition. Complementary mechanisms may be acting in AM plants. Perhaps part of the benefit of AM fungi at high salinity levels lies in the improved water relations, nutrient concentrations and absorption of water and nutrients from the soil solution of a different osmotic potential from that of the non-mycorrhizal-inoculated plants. Increased water resistance to water loss, together with osmotic adjustment, may enable salinized plants to maintain a better turgor maintainance during periods of drought. Studies on the effect of salinity on mycorrhizal-inoculated plants have shown that AM roots have higher Na concentrations but also have higher K concentrations and thus maintain a high K/Na ratio (Allen and Cunningham 1983; Poss et al. 1985). Nevertheless Pfeiffer and Bloss (1988) found decreased concentrations of Na in mycorrhizal-inoculated plants than in control plants. These results indicate that mechanisms other than increased P nutrition may be important in the effect of AM colonization under saline stress. Tolerance of salt stress and low water potential may involve cytoplasmic osmoregulation. Drought tolerance was expressed as a reduced CO_2 diffusion resistance. Such physiological plant parameters are also influenced by mycorrhizal colonization. The physiological mechanism(s) for the observed increased salt tolerance remains unclear.

Assessment of the spread of the mycorrhizal fungus and *Rhizobium* in saline soils revealed that the strain of *Rhizobium* was relatively more sensitive to salt than the *G. mosseae* isolate. Nodulation was severely reduced in the treatment irrigated with a saline solution of 28.8 dS m^{-1}, whereas mycorrhizal colonization was not altered under these conditions.

^{15}N enrichment of alfalfa shoots has been shown to diminish as P supply increases, indicating an improvement of N_2 fixation rates (Danso 1986). Our results indicate an indirect relationship between N_2 fixation and salt stress as expected.

Nodule formation was greater in AM-colonized plants than in plants in the P-fertilized treatments at each level of salinity. Using ^{15}N enrichment to assess nodule function

(N_2 fixation), the mycorrhizal treatment gave a greater improvement of N_2 fixation at the highest salinity level. In this treatment probably physiological mechanisms, related to photosynthetic rate or accumulation of solutes, must be involved in the mycorrhizal protective effect. In the other applied saline treatments (13.8, 22.2 and 28.8 dS m^{-1}), the mycorrhizal ^{15}N enrichment was similar to that in some P treatments. The greater numbers of nodules formed on mycorrhizal-inoculated plants than on plants in the P application treatments provides evidence of a lack of correlation between the nodular tissue and its activity (N_2 fixation) in the mycorrhizal treatments. These observations suggest AM-inoculated plants are prevented from reaching their full N_2 fixation potential, agreeing with previously reported results (Azcón and Barea 1992). Indeed, nodulated and mycorrhizal-inoculated alfalfa showed lower N_2 fixation than P-amended plants. The reduced activity (N_2 fixation) per nodule cannot be caused by P limitation since levels of this nutrient were similar or higher in all the mycorrhizal treatments. Despite this fact, the highest N acquisition was seen in mycorrhizal-inoculated plants. Similar results have been found by other studies (Azcón et al. 1988; Barea et al. 1989). This report therefore confirms that mycorrhizal-inoculated plants have alternative mechanisms available to satisfy their N requirements. These findings can be interpreted as indicating the capability of the external mycelium of mycorrhizal fungi to use N from the soil, which is less available to non-AM-inoculated plants (Tobar et al. 1994a,b).

The decreasing Ca and Mg acquisition by plants associated with the AM fungus can be attributed to the role of this microorganism in preventing an excessive cation uptake, which affects the cation/anion balance in the plant (Azcón and Barea 1992). The differential nutrient uptake and translocation by the shoot of the mycorrhizal-inoculated plants enable adverse environmental conditions to be tolerated (Lambais and Cardose 1993).

Mycorrhizal fungi possess specific individual traits with respect to their host tolerance to stress (Ruiz-Lozano et al. 1995). The results from this study suggest that AM fungi can tolerate soil salt stress conditions, allowing the numbers of associated nodules to increase.

This report, therefore, confirms previous findings (Azcón and Barea 1992) which suggest that AM-colonized plants have available alternative mechanism to satisfy their nutritive requirements in stress situations and in disturbed ecosystems in which growth conditions limit either nodulation or nodule activity.

Acknowledgements We thank the Joint FAO/IAEA Division, United Nations, Vienna, for supplying us with the ^{15}N-labelled fertilized and for the ^{15}N analysis. The authors would like to thank Dr. Barea for his stimulating discussion of this manuscript. The work was supported by CICYT Spain (Project AGR 91-0605-C02/01).

References

Allen MF, Boosalis MG (1983) Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. New Phytol 93:67—76

Allen EB, Cunningham GL (1983) Effects of vesicular-arbuscular mycorrhizae on *Destichlis spicata* under three salinity levels. New Phytol 93:227–236

Anon (1974) Technicon autoanalyzer-continuous-flow analytical instrument. Technical Publication UA4, 010A00

Azcón R, Barea JM (1992) Nodulation, N_2 fixation (^{15}N) and N nutrition relationships in mycorrhizal or phosphate-amended alfalfa plants. Symbiosis 12:33–41

Azcón R, El-Atrash F, Barea JM (1988) Influence of mycorrhiza vs. soluble phosphate on growth, nodulation, and N_2 fixation (^{15}N) in alfalfa under different levels of water potential. Biol Fertil Soils 7:28–31

Barea JM, Azcón R, Azcón-Aguilar C (1989) Time course of N fixation in the field by clover growing alone or in mixture with ryegrass to improve pasture productivity and inoculation with vesicular-arbuscular mycorrhizal fungus. New Phytol 112:399–404

Barret-Lennard EG, Malcolm CV, Stern WR, Wilkins SM (1986) Forage and fuel production from salt-affected wasteland. Elsevier, New York

Champagnol F (1979) Relationships between phosphate nutrition of plants and salt toxicity. Phosph Agric 76:34–43

Danso SKA (1986) Estimation of N_2 fixation by isotope dilution: on appraisal of techniques involving ^{15}N enrichment and their application – comments. Soil Biol Biochem 18:243–244

Dixon RK, Rao MV, Garg VK (1993a) Inoculation of *Leucaena* and *Prosopis* seedlings with *Glomus* and *Rhizobium* species in saline soil: rhizosphere relations and seedlings growth. Arid Soil Res Rehabil 7:133–144

Dixon RK, Rao MV, Garg VK (1993b) Salt stress affects in vitro growth and in situ symbioses of ectomycorrhizal fungi. Mycorrhiza 3:62–68

Fiedler R, Proksch G (1975) The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis: A review. Anal Chem Acta 78:1–62

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Hirrel MC, Gerdemann JW (1978) Improved salt tolerance in bell pepper by two vesicular-arbuscular (VA) mycorrhizal fungi. Agron Abst 140%141

Lambais MR, Cardoso EJBN (1988) Avaliaçao da germinaçao de esporos de fungos micorrízicos vesiculo-arbusculares e da colonizaçao micorrízica de *Stylosanthes gianensis* in solo acido e distrópico. R Bras C Solo 12:249–255

Mass EV, Nieman RH (1978) Physiology of plant tolerance to suboptimal land conditions. ASA Spec Pub 32:343–

Ojala JC, Jarrell WM, Menge JA, Johnson ELV (1983) Influence of mycorrhizal fungi on the mineral nutrition and yield of onion in saline soil. Agron J 75:255–259

Pfeiffer CM, Bloss HE (1988) Growth and nutrition of guayule (*Parthenium argentatum*) in a saline as influenced by vesicular-arbuscular mycorrhiza and phosphorus fertilization. New Phytol 108:315–321

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:159–161

Poss JA, Pond E, Menge JA, Jarrel WM (1985) Effects of sodium chloride on mycorrhizal onion and tomato in soil with and without additional phosphate. Plant and Soil 88:307–319

Reddell P, Foster RC, Bowen GD (1986) The effects of sodium chloride on growth and nitrogen fixation in *Casuarina obesa* Mig. New Phytol 102:397–408

Rosendahl CN, Rosendahl S (1991) Influence of vesicular-arbuscular mycorrhizal fungi (*Glomus* spp.) on the response of cucumber (*Cucumis sativus* L.) to salt stress. Environ Exp Bot 31:313–318

Ruiz-Lozano JM, Azcón R, Gomez M (1995) Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: Physiological and nutritional plant responses. Appl Environ Microbiol 61:456–460

Sprent JL (1986) Nitrogen fixation in a sustainable agriculture. Biol Agric 1:153–160

Tobar RM, Azcón R, Barea JM (1994a) The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorrhizae. Mycorrhiza 4:105–108

Tobar RM, Azcón R, Barea JM (1994b) Improved nitrogen uptake and transport from ^{15}N-labelled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. New Phytol 126:119–122