THE PHYSIOLOGICAL RESPONSES OF SALINITY STRESSED TOMATO PLANTS TO MYCORRHIZAL INFECTION AND VARIATION IN RHIZOSPHERE CARBON DIOXIDE CONCENTRATION.

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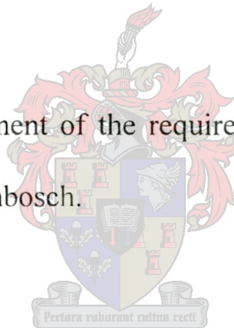
Thesis presented in partial fulfillment of the requirements for the degree of Master of Science at the University of Stellenbosch.



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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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ABSTRACT

This investigation was undertaken to determine whether elevated concentrations of dissolved inorganic carbon (DIC) supplied to plant roots could improve plant growth and alleviate the effects of salinity stress on tomato plants infected with arbuscular mycorrhizae. *Lycopersicon esculentum* cv. F144 seedlings were grown in hydroponic culture (pH 5.8) with 0 and 75 mM NaCl and with or without infection with the fungus *Glomus mosseae*. The root solution was aerated with ambient CO_2 (360 ppm) or elevated CO_2 (5 000 ppm) concentrations. The arbuscular and hyphal components of mycorrhizal infection as well as the percentages total infection were decreased or increased according to the variation in seasons. The plant dry weight of mycorrhizal plants was increased by 30% compared to non-mycorrhizal plants at elevated concentrations of CO_2, while the dry weight was decreased by 68% at ambient CO_2 concentrations. Elevated CO_2 also stimulated the growth of the mycorrhizal fungus. Elevated CO_2 increased the plant dry weight and stimulated fungal growth of mycorrhizal plants possibly by the provision of carbon due to the incorporation of HCO_3^- by PEPc. Plant roots supplied with elevated concentrations of CO_2 had a decreased CO_2 release rate compared to roots at ambient CO_2. This decrease in CO_2 release rate at elevated CO_2 was due to the increased incorporation of HCO_3^- by PEPc activity. Under conditions of salinity stress plants had a higher ratio of NO_3^-: reduced N in the xylem sap compared to plants supplied with 0 mM NaCl. Under salinity stress conditions, more NO_3^- was transported in the xylem stream possibly because of the production of more organic acids instead of amino acids due to low P conditions under which the plants were grown. The NO_3^- uptake rate of plants increased at elevated concentrations of CO_2 in the absence of salinity because the HCO_3^- could be used for the production of amino acids. In the presence of salinity, carbon was possibly used for the production of organic acids that diverted carbon away from the synthesis of amino acids. It was concluded that mycorrhizas were beneficial for plant growth under conditions of salinity stress provided that there was an additional source of carbon. Arbuscular mycorrhizal infection did not improve the nutrient uptake of hydroponically grown plants.

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UITTREKSEL

In hierdie studie was die effek van verhoogde konsentrasies opgeloste anorganiese koolstof wat aan plant wortels verskaf is, getoets om te bepaal of dit die groei van plante kan verbeter asook of sout stres verlig kon word in tamatie plante wat met arbuskulêre mikorrhizas geïnfekteer was. *Lycorpersicon esculentum* cv. F144 saailinge was in water kultuur gegroei (pH 5.8) met 0 en 75 mM NaCl asook met of sonder infeksie met die fungus *Glomus mosseae.* Die plant wortels was bespuit met normale CO_2 (360 dele per miljoen (dpm)) sowel as verhoogde CO_2 (5 000 dpm) konsentrasies. Die arbuskulere en hife komponente, sowel as die persentasie infeksie was vermeerder of verminder na gelang van die verandering in seisoen. Die plant droë massa van mikorrhiza geïnfekteerde plante by verhoogde CO_2 konsentrasies was verhoog met 30% in vergelyking met plante wat nie geïnfekteer was nie, terwyl die droë massa met 68% afgeneem het by gewone CO_2 konsentrasies. Verhoogde CO_2 konsentrasies het moontlik die plant droë massa en die groei van die fungus verbeter deur koolstof te verskaf as gevolg van die vaslegging van HCO_3^- deur die werking van PEP karboksilase. Plant wortels wat met verhoogde CO_2 konsentrasies bespuit was, het 'n verlaagde CO_2 vrystelling getoon in vergelyking met die wortels by normale CO_2 vlakke. Die vermindering in CO_2 vrystelling van wortels by verhoogde CO_2 was die gevolg van die vaslegging van HCO_3^- deur PEPk aktiwiteit. Onder toestande van sout stres, het plante 'n groter hoeveelheid NO_3^- gereduseerde N in die xileemsap bevat in vergelyking met plante wat onder geen sout stres was nie, asook meer NO_3^- was in die xileemsap vervoer moontlik omdat meer organiese sure geproduseer was ten koste van amino sure. Dit was die moontlike gevolg omdat die plante onder lae P toestande gegroei het. Die tempo van NO_3^- opname was verhoog onder verhoogde CO_2 konsentrasies en in die afwesigheid van sout stres omdat die HCO_3^- vir die produksie van amino sure gebruik was. In die teenwoordigheid van sout was koolstof moontlik gebruik om organiese sure te vervaardig wat koolstof weggeneem het van die vervaardiging van amino sure. Daar is tot die slotsom gekom dat mikorrhizas voordelig is vir die groei van plante onder toestande van sout stres mits daar 'n addisionele bron van koolstof teenwoordig is. Arbuskulere mikorrhiza infeksie het 'n geringe invloed gehad op die opname van voedingstowwe van plante wat in waterkultuur gegroei was.

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LIST OF ABBREVIATIONS

AM	Arbuscular mycorrhizae
DIC	Dissolved inorganic carbon
LAR	Leaf area ratio
LSD	Least significant difference
LWR	Leaf weight ratio
NR	Nitrate reductase
PEPc	Phosphoenolpyruvate carboxylase
SE	Standard error

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THE PHYSIOLOGICAL RESPONSES OF SALINITY STRESSED TOMATO PLANTS TO MYCORRHIZAL INFECTION AND VARIATION IN RHIZOSPHERE CO_2 CONCENTRATION.

1.1 GENERAL INTRODUCTION

Salinity stress is a common agricultural problem in semi-arid zones. As the soil dries or as salt levels build up, both water potential and osmotic potential in the soil decline, thus affecting plant growth and physiological processes (Hsiao, 1986). Lewis *et al.* (1989) identified ionic and osmotic effects as two major causes of salinity stress. The ionic effects include the interference with nutrient uptake and the transport of ions within the plant (Hawkins and Lewis, 1993). The osmotic effects are associated with the lack of cell wall extension and cellular expansion that cause growth to cease (Termaat and Munns, 1986). Salinity stress decreases plant growth by interfering with plant processes such as mineral uptake (Kleinkopf and Wallace, Halperin *et al.*, 1997 and Ojala *et al.*, 1983), respiration (Schwarz and Gale, 1981), photosynthesis (Papp *et al.*, 1983) and water relations (Hsiao, 1986). Salinity stress could be alleviated by the addition of DIC to the root zone or by forming a symbiotic association with arbuscular mycorrhizas.

The term "mycorrhizae" refers to the mutualistic symbiosis between fungi and plant roots (Azcon-Aguilar and Bago, 1994). The fungus may assist the plant with the uptake of nutrients such as N and P (Johansen *et al*, 1994, Powell and Bogyaraj, 1984), while the plant supplies the mycorrhizae with carbohydrates (Wright *et al*, 1998). Mycorrhizal fungi have also been reported to alleviate the effects of salinity stress on plants and increase plant growth. Duke *et al*. (1986) and Ojala *et al*. (1983) reported that mycorrhizas alleviated salinity stress by improving P uptake in plants, while Ruiz-Lozano *et al*. (1996) reported that mycorrhizas alleviated salinity stress by improving transpiration, photosynthetic carbon dioxide assimilation rates, stomatal conductance and water use efficiency.

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Roots and soil micro-organisms produce CO_2 through respiration which accumulates in the rhizosphere at concentrations normally between 2 000 and 5 000 µmol mol $^{-1}$ (Norstadt and Porter, 1984). In artificial culture systems aerated with ambient air, the concentrations of dissolved inorganic carbon (DIC) in the rhizosphere in the form of CO_2 and HCO_3^- is often less than 1 000 µmol mol $^{-1}$ (Cramer *et al.*, 1999). Elevated concentrations of DIC in the rhizosphere (5 000 µmol mol $^{-1}$) were shown to increase the growth of plants (Vapaavuori and Pelkonen, 1985 and Bialczyk *et al.*, 1994), especially in plants grown under conditions of salinity stress (Cramer and Lips, 1995).

It was reported by Cramer and Lips, (1995) that elevated concentrations of DIC supplied to the root zone improved plant growth under conditions of salinity stress. Poss *et al.* (1996) and Duke *et al.* (1986) reported that arbuscular mycorrhizal fungi improved plant growth under conditions of salinity stress due to improved nutrient uptake. In this study, it was hypothesised that the combination of arbuscular mycorrhizas and elevated concentrations of DIC in the root zone could alleviate the effects of salinity stress on hydroponically grown tomato plants. Futhermore, CO_2 was reported to stimulate the growth of the arbuscular mycorrhizal fungus (Bécard and Piché, 1989). The hypothesis that DIC could stimulate the growth of the mycorrhizal fungus and thereby improve plant growth and alleviate salinity stress was also tested. Cramer *et al.* (1995) reported that elevated DIC concentrations in the root zone increased the growth of salinity stressed plants by improved NO_3^- uptake and the provision of C-skeletons, for amino acid or organic acid synthesis. In this study, it was tested if elevated concentrations of CO_2 would have a similar positive effect on plant growth by increasing NO_3^- uptake as well as the production of DIC incorporation products. Johansen *et al.* (1994) concluded that mycorrhizal hyphae assisited plants with the uptake of NO_3^-, thereby improving

plant growth, although in the absence of salinity stress. Therefore, the possibility was tested that arbuscular mycorrhizas could improve plant growth and alleviate salinity stress by improved NO_3^- uptake. These hypotheses were tested by growing tomato seedlings in hydroponic culture with 360 and 5 000 ppm CO_2. Plants were grown with 0 and 75 mM NaCl in the absence and presence of the mycorrhizal fungus *Glomus mosseae*.

The following characteristics were measured:

(1) Biomass attributes such as plant dry weight, relative growth rate, moisture contents, shoot: root ratio, leaf area and specific leaf area.

(2) Proline concentrations and water potentials of plants as an indication of salinity stress.

(3) Respiration rate of plant roots was measured as O_2 consumption and CO_2 release and the Rq values were determined.

(4) The NO_3^- uptake rate, nitrate reductase (NR) activity, xylem sap NO_3^- and reduced N concentrations and total N concentration.

(5) Arbuscular, vesicle, hyphal and total infection of mycorrhizal plants.

1. 2 LITERATURE REVIEW

In this section, the effects of salinity stress on physiological processes, in particular mineral nutrition and respiration have been addressed. These processes were chosen because the focus of this study was on the effect of DIC and salinity on plant roots and mineral uptake and root respiration would be directly affected. The role of the compatible solute proline was discussed because it gave a measurement of the adaptability of plants to salinity stress. Aspects of the arbuscular mycorrhizal (AM) symbiosis such as the effects of AM on salinity stress, N uptake and the cost of maintenance to the plant was reported on. These aspects were discussed because it could give an indication if mycorrhizas are beneficial to plant growth. The provision of DIC

to the root zone was discussed in terms of the effect of DIC on plant growth under salinity stress conditions and the influence of DIC on the growth of the AM fungus that were implicated as important beneficial effects of DIC.

1.2.1 Physiological effects of salinity stress

1.2.1.1 Mineral nutrition

Lewis *et al.*, 1989 reported that growth reductions under salinity stress conditions were commonly attributed either to ionic toxicity or to a low osmotic potential. Hawkins and Lewis (1993) concluded that NO_3^- uptake was non-competitively inhibited by salinity and Peuke and Jescke (1999) suggested that NO_3^- uptake was inhibited by an osmotic effect.

Saline toxicity could be caused by the Na^+ as well as Cl^- ions. Romero-Aranda *et al.* (1998) concluded that physiological disturbances caused by salinity in citrus trees could be linked to Cl^- accumulation in plant leaves. Kinraide (1999) reported that the Na^+ ions could cause growth limitations by the displacement of Ca^{2+} at the plasma membrane root surface of cells. Salinity stress decreased NO_3^- uptake, and also interfered with the reduction and assimilation of NO_3^- within the plant (Cramer & Lips, 1995). Peuke *et al.* (1996) reported that a mild stress treatment of 40 mM NaCl decreased the concentrations of both NO_3^- and NH_4^+ in *Ricinus communis* L. and lowered the percentages of the inorganic component relative to total nitrogen in the xylem sap. The mild salt treatment resulted in 58% of nitrate being reduced in the root compared to 33% root nitrate reduction in control plants.

1.2.1.2 Respiration

The growth, maintenance of biomass and transport of nutrients are three of the major processes that occur in the plant root requiring respiratory energy (van der Werf *et al.*, 1988). Energy is

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required for the maintenance of chemical and electrochemical gradients across membranes and for the turnover of cellular constituents such as proteins (van der Werf *et al.*, 1988) as well as the uptake of ions. The uptake of ions could account up to 60% of the root respiration under normal conditions (van der Werf *et al.*, 1988).

The rate of respiration could increase as a consequence of additional energetic costs. Growth expressed as an increase in dry weight could be reduced even at low levels of salinity (Lewis *et al.*, 1989). It was proposed that this reduced growth in the plant was caused by the diversion of assimilate resources from growth to maintenance respiration (Greenway, 1973). These assimilates would be needed to meet the challenge of compartmentation and excretion of salt and for repair of cellular damage caused by the presence of salt (Penning De Vries, 1975). Schwarz and Gale (1981) reported that at low levels of salinity, maintenance respiration increased with an increase in salt concentration equivalent to 0.1 MPa (equivalent to 20 mM NaCl) osmotic potential. The increased maintenance respiration at higher levels of salinity stress was suggested to be an indication of the commencement of salt damage. Schwarz and Gale (1981) working with *Phaseolus vulgaris*, *Xanthium strumarium*, *Zea mays* and *Atriplex halinus* concluded that part of the reduction of growth caused by salinity stress might be explained by the diversion of assimilates from anabolic to catabolic processes.

Blacquière and Lambers (1981) reported that a concentration of 50 mM NaCl did not interfere with respiration of *Plantago coronopus,* although the net photosynthesis was slightly decreased by saline conditions. The rates of dark respiration of the shoots and the roots were the same under saline and non-saline conditions and these authors concluded that the extra energy requirements for growth in this saline environment were negligible in terms of the total energy costs of the plant for growth and maintenance processes in a non-saline environment. These

different results reported by Schwarz and Gale (1981) and Blacquière and Lambers (1981) could be the effect of different salt concentrations on respiration as well as the different plant species that were used in the two studies.

1.2.2 Mechanisms of tolerance to salt stress

1.2.2.1 Compatible solutes

One of the most common responses of organisms to salinity stress and water deficit is the production and accumulation of compatible solutes. These are osmotically active neutral organic compounds such as sugars (polyols), certain amino acids, and quaternary ammonium compounds (Taylor, 1996). Proline is considered as the principal solute in tolerance to osmotic stress, acting as a compatible osmolyte. The maintenance of appropriate water potential during a water deficit can be achieved by osmotic adjustment. A reduction in the cellular water potential to lower than the external water potential, resulting from a decrease in osmotic potential allows water to continue to move into the cell. The osmotic potential inside the cell is lowered by the accumulation in the cytosol of compatible solutes such as proline (Yoshiba *et al.*, 1997). Hare and Cress (1997) proposed an alternative role for proline synthesis, that it could be a mechanism to remove excess H^+ and thereby prevent a depression in the respiration of stressed plants.

1.2.3 Arbuscular Mycorrhizae

1.2.3.1 The effect of salinity on AM infection

The term "mycorrhizae" refers to the mutualistic symbiosis between fungi and roots (Azcon-Aguilar & Bago, 1994). The formation of arbuscular mycorrhizas can be distinguished into "primary" infection which is the first entry into the root by the fungus and "secondary" infection, which occurs after fungal hyphae have ramified from sites of initial colonization

(Wilson, 1984). Primary infection is dependent on spore germination and the growth of the hyphae through the soil as well as the entry into the plant (Bowen, 1987). Secondary infection is influenced by the physiology of the host plant, because most of the energy for the spread of hyphae is obtained from photosynthate translocated from the plant to the fungus, either through the arbuscular interface (Blee and Anderson, 1998) or via the internal hyphae.

Data on the effect of salinity on primary infection by mycorrhizas indicated that spore germination could be inhibited by increasing concentrations of NaCl (Hirrel, 1981). Estaun (1989) and Juniper and Abbott, (1991) reported that the growth of hyphae of arbuscular mycorrhizal species *Glomus mosseae, Acaulospora trappei, Scutellospora calospora* and *Gigaspora decipiens* was inhibited by NaCl in the growth medium. Salt in the growth medium not only induced changes in the length but also in other morphological properties of hyphae. High concentrations of $CaCl_2$, KCl and NaCl in the growth medium shortened primary germ tubes and stimulated lateral branching of hyphae in *Gigaspora margarita* (Hirrel, 1981). NaCl reduced the number of arbuscules and vesicles as well as the colonization with increasing concentrations of NaCl (Pfeiffer and Bloss, 1988).

The photosynthetic activity of the plant would be expected to affect the carbohydrate status of the roots and the amount of mycorrhizal colonization (Wright *et al*., 1998). To support the mycorrhizal symbiosis, the host plant has to transfer C to the mycorrhizal fungus that could amount to between 4 and 20% of the total net C fixed by the plant (Pang and Paul, 1980).

1.2.3.2. Effects of AM on drought and salinity responses to plants

Ojala *et al*. (1983) and Duke *et al.* (1986) reported that AM fungi improved plant growth and salt tolerance in salinity stressed onion plants by improvement of the P nutrition. Although

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improved P nutrition was reported to be the most likely mechanism by which yields were improved, mycorrhiza could improve plant growth and salt tolerance in other ways, such as through improved water uptake. Poss *et al.* (1995) suggested that arbuscular mycorrhizal fungi might influence plant hormones such as cytokinin production and thereby improve water uptake. Hyphae of mycorrhizal fungi often extend 7 cm or more beyond the rhizosphere into soil and could assist with nutrient uptake as an extension of the absorptive surface area of the roots due to their ability to explore more soil volume (Ruiz-Lozano & Azcon, 1995). In addition, mycorrhizas could enabled plants to maintain leaf turgor and stomatal conductance at greater tissue water deficits and lower leaf and soil water potentials when compared to non-mycorrhizal plants (Ruiz -Lozano *et al.*, 1996) due to better access to water. The proline concentration of lettuce plants was increased under conditions of salinity stress, although mycorrhizal plants grown with salinity had a lower proline concentration compared to non-mycorrhizal plants (Ruiz - Lozano *et al.*, 1996). These authors suggested that the mycorrhizas were better able to protect the colonised plants against salinity stress by influencing the physiological processes of increased carbon dioxide exchange rate, transpiration, stomatal conductance and water use efficiency (WUE). They proposed that plants infected with mycorrhizas needed to accumulate less proline because the mycorrhizas protected the plants against salinity stress by increased transfer of water from mycorrhizal hyphae to the plant (Ruiz- Lozano and Azcón, 1995). AM fungal species such as *Glomus deserticola* and *G. etunicatum* were more efficient in alleviating stress by being more resistant to stress than *G. caledonium* and *G. ocultum*. Characteristics such as photosynthetic activity, WUE, transpiration and stomatal conductance were increased in lettuce plants following a progressive drought stress period (Ruiz-Lozano *et al.*, 1995). From the results it was concluded that the ability of *G. deserticola* and *G. etunicatum* to protect the host plant against progressive drought

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stress could be ascribed to physiological as well as nutritional mechanisms such as increased P and K uptake (Ruiz-Lozano *et al.*, 1995).

Bryla & Duniway (1997) observed that mycorrhizas did not play a significant role in improving the drought tolerance of wheat plants. The mycorrhizal infection did not affect changes in the leaf water status when plants were exposed to increasing levels of drought stress and had little effect on drought tolerance when drought stress was very severe. The mycorrhizas did not help the wheat plants to extract water from the soil, even when the soil moisture conditions were very dry. They found that P acquisition was significantly enhanced by mycorrhizal infection in wheat and concluded that plants infected with mycorrhizas had more P in their plant tissue than uninfected plants and consequently that inoculation could improve plant resistance to drought stress by improved P nutrition.

1.2.3.3 AM and N uptake

A young, elongating, uninfected root growing through the soil will normally absorb NO_3^- from a wide radius, because of the rapid movement of NO_3^- to the root by diffusion and mass flow (Bowen & Smith, 1981). Nitrate supply would not be limiting when NO_3^- rapidly moves towards the root and mycorrhizal effects on absorption would be expected only if the fungi had a higher absorbing power than the root. However, with very low soil nitrate levels and declining soil moisture, NO_3^- absorption could become diffusion-limited and in such cases, absorption by hyphae extending into the soil may enhance NO_3^- uptake (Bowen & Smith, 1981). Tobar *et al.* (1994) reported that when the movement of NO_3^- ions in the soil solution was limited by drought stress conditions, the ^{15}N in plant tissues derived from the labelled $^{15}NO_3^-$ was significantly higher in mycorrhizal plants. The external mycelium of arbuscular mycorrhizas took up and transported NO_3^- and thus improved plant nutrition. In contrast,

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Hawkins *et al.* (1999) reported that mycorrhizas did not increase the N or P concentration of wheat plants grown in hydroponic culture. The authors concluded that this result was probably the consequence of the unlimited diffusion of nutrients in hydroponics and this would eliminate the benefit of mycorrhizas.

1.2.3.4 The cost of maintaining the AM fungus

As obligate symbionts, mycorrhizas require organic compounds from their hosts. The fungus could improve water and nutrient (P and N) uptake of the plant, but in turn it could make an impact on the organic carbon supplies of the plant. Buwalda and Goh (1982) suggested that the depletion of the photosynthetically derived C supply was probably the mechanism by which mycorrhizas depress growth. Such depressions may result from the diversion of carbon from the host root to external mycorrhizal structures. Jacobsen and Rosendahl (1990) reported that the carbon drain by the fungal tissues of AM was large enough to significantly affect the carbon distribution within the plant. The C allocation to the external hyphae was about 4% of the photoassimilated ^{14}C and it was estimated that the fungal biomass of the VA mycorrhizas and its respiration consumed 20% of the photo-assimilated ^{14}C. Baas *et al.* (1989) also reported that root respiration rates per unit root dry weight was on the average 76 to 79% greater in mycorrhizal compared to non-mycorrhizal plants. Mycorrhizal plants used more daily photosynthate for respiration than non-mycorrhizal plants. It was estimated that 87% of the increase in root respiration due to mycorrhizal infection could be ascribed to respiratory costs of the AM symbiosis for its own respiration, whereas only 13% of the increase could be ascribed to the greater ion uptake rates (Baas *et al.*, 1989). Hawkins *et al.* (1999) reported that mycorrhizal colonisation in hydroponically grown wheat plants resulted in a 32% and 61% increase in CO_2 release rate and Rq value, while O_2 consumption was not significantly affected.

Hawkins *et al.* (1999) suggested that the 32% increase in CO_2 release rate from mycorrhizal roots was due to fungal respiration.

1.2.4 Elevated root zone DIC

1.2.4.1 The effect of DIC on plant growth

In the roots DIC in the form of CO_2 and HCO_3^- (CO_3^{-2}-$<$ 0.1% of DIC if pH $<$ 7.5) (Cramer *et al.*, 1999) is first metabolised into organic acids and later into proteins and starch (Vuorinen and Kaiser, 1997). DIC in the form of HCO_3^- at a concentration of 5.68 mM HCO_3^- increased the dry matter production of tomato plants (Bialczyk *et al.*, 1994). Vapaavuori and Pelkonen (1985) also reported an increase in the dry matter production of willow plants in hydroponic culture with the addition of HCO_3^- to the root medium at concentrations of between 0.015 and 1.5 mM HCO_3^-.

1.2.4.2. The effect of DIC on plant growth under salinity stress conditions.

The dry weight of hydroponically grown tomato plants were increased by elevated concentrations of DIC supplied with 5 000 ppm CO_2 under salinity stress conditions (Cramer and Lips, 1995). Plants grown under conditions of salinity stress and enriched DIC also had higher rates of NO_3^- uptake and translocated more NO_3^- and reduced N in the xylem sap than plants supplied with ambient DIC concentrations (Cramer and Lips, 1995). In salinity stressed plants, the products of DIC assimilation were preferentially diverted into amino acid synthesis, while more organic acids were found in non-salinised plants (Cramer *et al.*, 1995). The authors concluded that enriched DIC had a positive influence on the growth of salinity stressed plants because it provided a source of anapleurotic carbon for amino acid synthesis in the roots of plants forced to assimilate NO_3^- in the root due to salinity.

The root NR activity *in vivo* was higher in plants treated with 200 mM NaCl than in control plants (Cramer *et al.*, 1995). Elevated DIC could cause partial acidification of the root cytoplasm through CO_2 hydration to HCO_3^- and thereby stimulate NRA (Mengel *et al.*, 1983). The roots of plants grown under saline conditions also had significantly higher PEPc activities *in vitro* than control plants (Cramer *et al.*, 1999). These authors proposed that increased root N assimilation in salinity treated plants supplied with elevated DIC would require an increased provision of C skeletons for the assimilation of NH_4^+ into amino acids and this would require higher anapleurotic PEPc activity.

1.2.4.3 The effect of DIC on AM fungal growth

DIC could serve as an anapleurotic source of carbon in fungi (Griffin, 1994) by the metabolic utilisation of CO_2 being incorporated into the TCA cycle, replacing dicarboxylic acids that are used for amino acid synthesis (Gitterman and Knight, 1952). CO_2 could play a role in the stimulation of growth of the AM fungus. Saif (1984) reported an increase in the percentage of root length infected and the number of vesicles in the roots of *Eupatorium odoratum L*, *Guizotia abyssinica* and *Sorghum bicolor* grown with concentrations of 0.5% CO_2. Bécard and Piché (1989) also reported that CO_2 at 0.5% was able to stimulate the growth of *Gigaspora margarita* spores, in *in vitro* studies using root tissue. The possible mechanism of CO_2 stimulation of hyphal growth of AM is the utilisation of CO_2 in the synthesis of lipids (Sasaki *et al.*, 1995).

In summary, salinity stress has a negative effect on plant growth, by influencing N uptake and assimilation and in some instances root respiration. Proline accumulation could be a possible mechanism to alleviate salinity stress in plants. Arbuscular mycorrhizal fungi could also alleviate salinity stress in plants by improving N or P uptake or through modifying the

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photosynthetic activity, WUE, transpiration rate and stomatal conductance. In turn, the fungus could use a substantial amount of the carbon resources of the host plant, perhaps depressing growth in some circumstances. Elevated DIC could play a positive role in increasing plant biomass, even under salinity stress conditions. Elevated DIC could assist the plant with NO_3^- uptake under salinity stress conditions and DIC could stimulate fungal growth at concentrations of less than 1% CO_2.

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2 THE GROWTH RESPONSES OF SALINITY STRESSED TOMATO PLANTS TO MYCORRHIZAL INFECTION AND VARIATION IN RHIZOSPHERE CO_2 CONCENTRATION.

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Key Words: salinity, arbuscular mycorrhizas, Lycopersicon esculentum, CO_2

2.1 Abstract

This investigation was undertaken to determine whether elevated concentrations of dissolved inorganic carbon in the root medium could improve plant growth and alleviate the effects of salinity stress on tomato plants infected with arbuscular mycorrhizae. *Lycopersicon esculentum* cv. F144 seedlings were grown in hydroponic culture (pH 5.8) with 0 and 75 mM NaCl and with or without infection with the fungus *Glomus mosseae*. The root solution was aerated with either ambient CO_2 (360 ppm) or elevated CO_2 (5 000 ppm). CO_2 as well as salinity had a stimulatory effect on mycorrhizal growth. Elevated concentrations of CO_2 increased the dry weight of mycorrhizal plants by 30% compared to non-mycorrhizal plants, but the dry weight of mycorrhizal plants was decreased by 68% at ambient concentrations of CO_2. Elevated concentrations of CO_2 increased the plant dry weight and stimulated mycorrhizal growth possibly by the provision of carbon due to the incorporation of DIC via PEPc. In the absence of elevated CO_2, the mycorrhizal fungus possibly competed with the plant for available carbon and therefore there was a decrease in plant dry weight. The leaf proline concentration of mycorrhizal plants was increased by 48% and 57% ,respectively, due to the presence of salinity stress. Salinity stress also decreased the total N concentration of plants. It was concluded that arbuscular mycorrhizas could be beneficial for plant growth, especially under salinity stress conditions in the presence of anapleurotic carbon.

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2.2 Introduction

Amongst the most important processes in plants that are inhibited by salinity stress are NO_3^- (Cramer *et al.* 1995) and NH_4^+ uptake (Lewis *et al.*, 1989). NO_3^--fed wheat and maize plants grew vigorously at 80 mM NaCl, while NH_4^+ fed plants showed signs of wilt and some plants even died (Lewis *et al.*, 1989). In maize and wheat plants, xylem transport of NO_3^- was inhibited under salinity stress conditions and N assimilation took place predominantly in the root at an energetic cost that resulted in restricted root growth (Lewis *et al.*, 1989).

A possible mechanism for the alleviation of salt stress could be the supplementation of dissolved inorganic carbon (DIC) to plant roots. Cramer and Lips (1995) reported that DIC enrichment resulted in a greater biomass accumulation in leaves and roots of hydroponically grown tomato plants supplied with salinity concentrations of 100 mM NaCl. Cramer and Lips (1995) proposed that root incorporation of DIC and re-fixation of respiratory CO_2 had a significant influence on plant growth and that DIC could assist the plant with N uptake under salinity stress conditions. Cramer *et al.* (1995) concluded that root based N assimilation could be linked to the incorporation of DIC into the roots as an anapleurotic source of carbon for amino acid synthesis.

DIC does not only serve as an anapleurotic source of carbon in roots, but also in fungi (Griffin, 1994). A potential avenue for the metabolic utilisation of CO_2 in the growth of fungi is the incorporation into the TCA cycle, replacing dicarboxylic acids that are used for amino acid synthesis (Gitterman and Knight, 1952; Griffin, 1994). CO_2 could thus stimulate the growth of AM fungi. Concentrations of up to 1% soil CO_2 supplied to the roots of *Eupatorium odoratum* stimulated fungal growth by increasing the root length infected and the number of vesicles of the host plant (Saif, 1984). The possible mechanism of CO_2 stimulation of hyphal growth of

AM could be the utilisation of CO_2 in the synthesis of lipids (Sasaki *et al.*, 1995). Furthermore, Bécard and Piché (1989) reported that elevated CO_2 could enhance the mobilisation of lipids as a source of carbon for hyphal growth. Since CO_2 was important in stimulating mycorrhizal growth, it could be speculated that elevated concentrations of CO_2 could play a vital role in stimulation of mycorrhizal growth in plants grown under salinity stress conditions.

Arbuscular mycorrhizal fungi form a mutualistic symbiosis with plant roots (Harley and Smith, 1983) and could assist plants with P uptake and possibly also with N uptake (Johansen *et al.*, 1994). AM played an important role in the alleviation of salinity stress in onion plants (Ojala *et al.*, 1983) by increasing P concentration when soil P was low (Poss *et al.*, 1985). However, Ruiz-Lozano *et al.* (1996) reported that the effects of fungi on salinity tolerance in lettuce plants could not be attributed to a difference in P concentration, since they found no significant differences in P content in their mycorrhizal and P fertilization treatments. Ruiz-Lozano *et al.* (1996) concluded that AM alleviated salinity stress in lettuce plants through increased photosynthetic CO_2 exchange rates, transpiration rates, stomatal conductances and water use efficiencies, rather than through improved P uptake.

In this investigation, the role of elevated DIC and AM infection in the alleviation of salinity stress and the improvement of growth/biomass of tomato plants were evaluated. The possibility that DIC could provide an additional source of C to the plant and stimulate AM infection, thus ameliorating salinity stress, was also investigated. This was tested by investigating the effects of DIC and AM on biomass characteristics, proline concentration and N concentrations which were measured in shoots and roots of plants grown with or without salinity stress.

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2.3 Materials and methods

2.3.1 Growth conditions

Seeds of *Lycopersicon esculentum* L. Mill F114 were germinated in pots (12.5 cm in diameter) of sterilised sand mixed with 10 g of live mycorrhizal inoculum of *Glomus mosseae* (supplied by Agricultural Genetics Co. LTD, UK). The inoculum consisted of chlamydospores and fragments of root in a clay support material that was placed directly under the seeds. The sand was sterilized in an autoclave for 4 h at 110°C under steam pressure at 200 kPa. Control plants received no inoculum, but were irrigated one week after germination with an extract (0.5 g inoculum ml^{-1} distilled H_2O) from the live inoculum. The extraction was performed using a 30 µm mesh to exclude *Glomus mosseae* spores (Abbott and Robson, 1979). The purpose of the treatment was to introduce microbes into the control plants that would be present in the live inoculum but excluding the mycorrhizas. Seedlings were watered to field capacity with Long Ashton nutrient solution (Hewitt, 1966) modified to contain 50 µM Na-phosphate and 0.1 mM MES. $NaNO_3$ was used as N source and the pH was maintained at 5.8 by adjusting with NaOH and HCl daily. The non-mycorrhizal plants also received benlate (Agricura) at a concentration of 0.057 g l^{-1} at field capacity and at 5 d intervals until the seedlings were transferred into hydroponics. The plants received 75 mM NaCl as salinity treatment initially supplied over a period of 2 d. The plants were grown in a greenhouse between November and December with a midday irradiance of *ca.*1000 µmol m^{-2} $s,^{-1}$ an average day/night temperature of 19/33°C and relative humidity of 60%.

After 5 weeks in the pots, the inoculated seedlings were transferred into 22 l hydroponic tanks after carefully rinsing the roots with deionised water. The hypocotyls of the plants were wrapped with foam rubber and the plants inserted through holes in the lids of the hydroponic tanks (six plants per tank). The nutrient solution was replaced every 5 d and the pH of the

medium was maintained at 5.8 by adjusting with NaOH and HCl daily. The CO_2 was supplied from a cylinder of industrial grade CO_2 and mixed with compressed air to obtain the appropriate CO_2 concentration. Air was bubbled into the lower opening of a 1 cm diameter tube that functioned as an airlift. This was done to circulate the nutrient solution and provide aeration and to prevent the mycorrhizal hyphae from being damaged by excessive solution movement. The CO_2 concentration was monitored continuously using an ADC Model 225 MK3 infra red gas analyzer (Analytical Development Corporation, Hoddesdon, UK) set up in absolute mode with a resolution of 10 ppm CO_2. Calibration was performed by mixing pure CO_2 with N_2 in a gas mixing syringe (Li-Cor Inc. Model 6 000-01, Lincoln, NE). The CO_2 concentrations were maintained at either 360 or 5 000 ppm.

2.3.2 Experimental design and statistical analysis

The experiment comprised 3 factors, with two levels each. The factors were AM inoculation (live or no inoculum), salinity (0 and 75 mM NaCl) and CO_2 (360 and 5000 ppm CO_2). Each treatment was replicated 6 times and all the plants of one treatment were grown in one tank. The effects of the treatment were tested using analysis of variance (ANOVA) followed by a post-hoc least significant difference (LSD) test performed using Statgraphics Vers.7.0. Prior to analysis of variance, percentage data were arcsine transformed (Zar, 1981).

2.3.3 Mycorrhizal infection

Root segments were excised and stored in 50% ethanol. Root segments were rinsed and cleared with 2.5% KOH at 110°C for 6 min at 110°C under steam pressure at 200 kPa in the autoclave. Afterwards, the KOH was rinsed from the segments and acidified with 1% HCl for at least 5 h. The roots were stained with 0.05% Analine blue in 70% acidified glycerol at 110°C for 5 min in the autoclave. Roots were cut into 1cm pieces and examined at x 400 magnification under a

light microscope. Infection was determined according to methods described by Brundrett *et al.* (1994) with some modifications. Brundrett *et al.* (1994) used the calculation: 100*[(q+r+s+t+u)/G)] to calculate the total infection, where p = no fungal structures, q = arbuscules, r = mycorrhizal vesicles, s = arbuscules and mycorrhizal vesicles, t = mycorrhizal hyphae but no arbuscules or mycorrhizal vesicles, u = hyphae not seen to be connected to arbuscules or mycorrhizal vesicles and G = (p+q+r+s+t+u).

In our calculation total infection was determined as = 100*[(q+r+u)/G). The components, s and t were not used as part of the calculation because no arbuscules and vesicles were observed together during the estimation and t was interpreted to fall in the same category as u.

2.3.4 Plant harvest

The fresh weight was determined immediately before transfer into hydroponics after blotting the roots dry. Plants were then harvested 10 d after transfer into hydroponic culture. The plants were divided into root, leaf and stem components and weighed to determine fresh weight. The leaf areas of the plants were measured with a portable leaf area meter (Li-cor, model LI-3000, Lambda Instruments Corporation, USA) and the water potential was determined with a pressure chamber (PMS instruments Co., Oregan USA). The roots were carefully blotted dry and a piece of root cut off, weighed and stored in a vial in 50% (v/v) ethanol for estimation of mycorrhizal infection. The remaining plant components were dried at 80°C for 48 h and weighed to determine the dry weight. RGR was calculated using the natural logs of the initial and final fresh weight of each plant.

2.3.5 Total N concentration and proline determination.

The oven-dried (48 h, 80°C) plant material of six replicates of each treatment was milled in a Wiley mill (A.H. Thomas, Philadelphia, Pa, USA) using a 60 mesh screen, for shoot and root

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(0.05 g) material. Material was digested in 35 cm long tubes with 4 ml of 3.4% (w/v) salicylic acid in 13.5 M sulphuric acid and a Kjedahl selenium catalyst tablet (Saarchem, Montague Gardens, South Africa). The samples were digested at room temperature for 2 h, 200°C for 1 h, 270°C for 1 h and 370°C for 1 h. The digest was diluted and assayed for NH_4^+ according to Solorzano (1969). Proline concentration was determined with freshly harvested plant leaves. Six replicates of each treatment were used to determine the proline concentration colourometrically using the ninhydrin method of Bates *et al*. (1972).

2.4 Results

2.4.1 Mycorrhizal infection

The arbuscular and total infections of the non-mycorrhizal plants were very low compared to the mycorrhizal plants. There were differences in arbuscular and hyphal infection of non-mycorrhizal plants at 5000 ppm CO_2, but these differences were relatively small and because infection of non-mycorrhizal plants was very low, non-mycorrhizal infection has not been discussed further. In all the AM treatments arbuscules were the largest component of infection while vesicles and hyphae made up less than 10% of infection (Figure 2.1). Mycorrhizal plants supplied with 0 mM NaCl had a higher percentage of vesicles and hyphae than plants supplied with 75 mM NaCl (Figure 2.1). At 0 mM NaCl, the arbuscular infection of plants were increased by 64% and the total infection by 43% at elevated concentrations of CO_2 compared to plants supplied with ambient CO_2 (Figure 2.1). Plants supplied with 360 ppm CO_2 had an increased arbuscular percentage of 50% and total infection of 22% respectively at 75 mM NaCl compared to plants at 0 mM NaCl. The total infection of mycorrhizal plants supplied with 360 and 5 000 ppm CO_2 was the same at 75 mM NaCl. At 0 mM NaCl, the percentage hyphal infection was increased in plants supplied with ambient concentrations of CO_2 compared to elevated CO_2.

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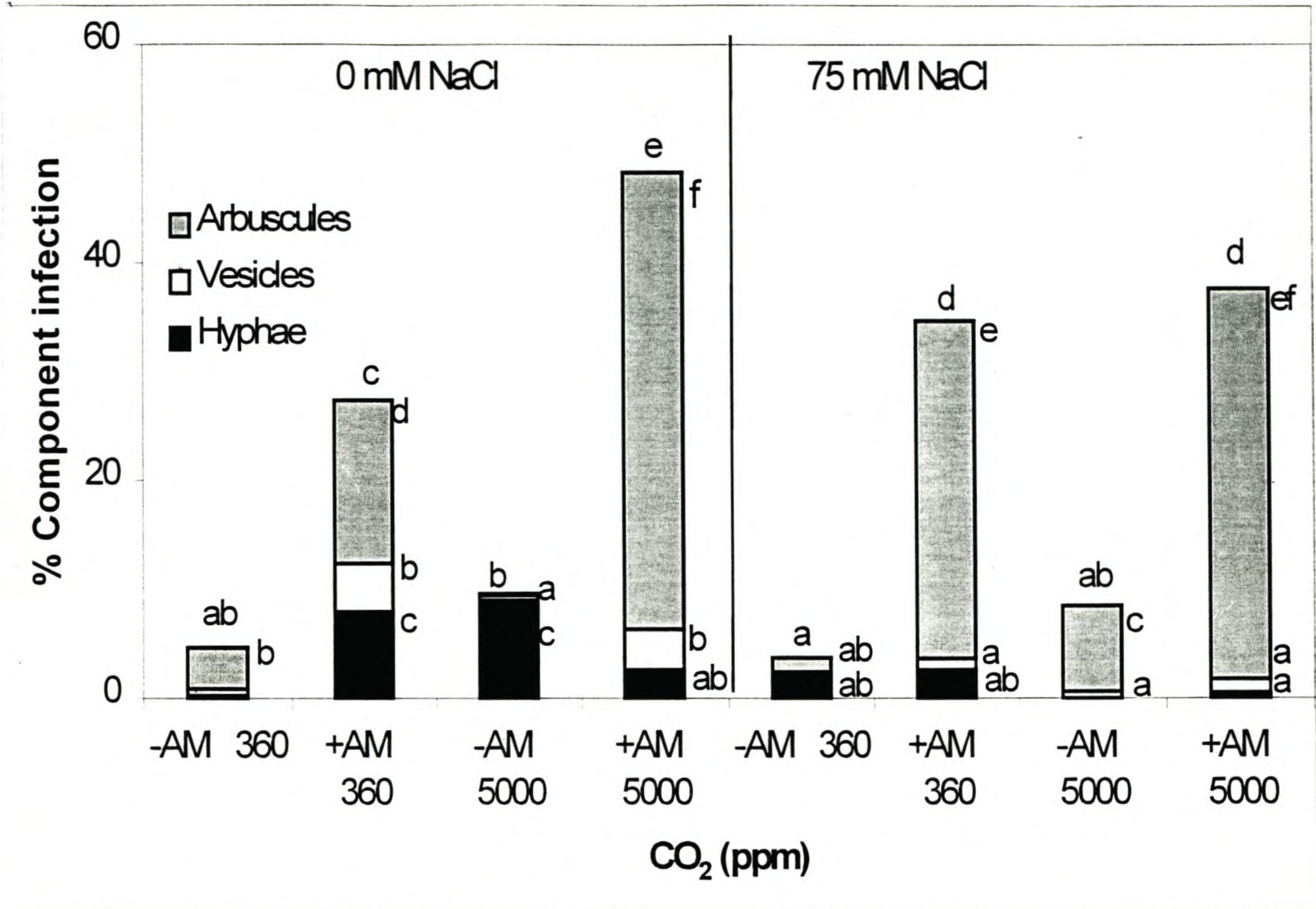

Figure 2.1: Effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 5 000 ppm CO_2 on the percentages arbuscular, vesicle and hyphal infections of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants (n=6). Dissimilar letters above the bars indicate significant differences ($P < 0.05$) between the means of total infection and letters next to the bars indicate arbuscular infection, and vesicle and hyphal infection where applicable determined from analysis of variance followed by Fisher's protected LSD test.

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Table 2.1: Sources of variation expressed as F-ratios for mycorrhizal infection, biomass characteristics, total N concentrations, shoot: root N content, leaf proline concentration and water potential of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants grown in hydroponic culture with either 0 or 75 mM NaCl and aeration of the root solution with either ambient (360 ppm) or enriched (5000 ppm) CO_2. Mycorrhizal infection data was arcsine transformed. The table represents the results of a 3-way ANOVA; total =40; *P ≤ 0.05, ns = not significant. The critical F value for df=40 at p < 0.05 = 4.05.

	Sources of variation						
	Factors			Interactions			
	CO_2	NaCl	AM	CO_2 x NaCl	CO_2 x AM	AM x NaCl	CO_2 x NaCl x AM
Mycorrhizal infection							
Arbuscules	18.4 *	10.4 *	340 *	0.19 ns	12.2 *	0.01 ns	38.9 *
Vesicles	0.01 ns	8.9 *	15.7 *	0.22 ns	0.46 ns	6.5 *	0.30 ns
Hyphae	17.6 *	0.22 ns	222.5 *	2.9 ns	0.13 ns	0.01 ns	3.7 ns
Total infection	17.4 *	4.3 *	345.9 *	0.32 ns	8.4 *	0.43 ns	32.4 *
Biomass characteristics							
Leaves	5.6 *	1.1 ns	3.2 ns	4.6 *	50 *	0 ns	1.1 ns
Stems	9.6 *	0.02 ns	3.1 ns	4.5 *	71.4 *	0.31 ns	5.0 *
Roots	4.2 *	8.2 *	0.03 ns	1.8 ns	69.6 *	3.0 ns	1.7 ns
Plants	8.5 *	1.6 ns	3.0 ns	5.3 *	78.5 *	0.29 ns	2.8 ns
Relative growth rate	58.6 *	15.5 *	25.5 *	52.4 *	109.8 *	2.9 ns	14.3 *
Shoot: Root ratio	2.2 ns	1.8 ns	7.4 *	1.7 ns	1.7 ns	0.95 ns	0.08 ns
Weight ratio Leaf	0.63 ns	0.83 ns	0 ns	0.16 ns	5.5 *	3.0 ns	1.4 ns
Stem	6.1 *	0.09 ns	5.2 *	2.3 ns	14.0 *	1.7 ns	2.2 ns
Root	3.6 ns	1.7 ns	7.4 *	1.6 ns	1.7 ns	0.55 ns	0.03 ns
Leaf area ratio	27.2 *	0.47 ns	29.6 *	26.1 *	50.5 *	50.3 *	4.2 *
Leaf area	0.04 ns	13.7 *	2.7 ns	0.58 ns	1.6 ns	32.1 *	2.2 ns
Specific leaf area	16.1 *	0.04 ns	19.9 *	16.1 *	28.9 *	29.2 *	2.1 ns
Moisture content Plant	0.44 ns	2.5 ns	0.38 ns	0.92 ns	7.2 *	0.42 ns	10.8 *
Mineral concentrations							
Total N Shoot	1.6 ns	15.3 *	1.9 ns	1.6 ns	32.2 *	0.5 ns	6.1 *
Root	2.3 ns	18.2 *	0.17 ns	2.9 ns	0.33 ns	0.03 ns	0.01 ns
Plant	5.3 *	40.9 *	1.06 ns	5.3 *	2.7 ns	5.4 *	1.8 ns
S:R N Content	1.9 ns	0.10 ns	1.08 ns	3.7 ns	12.4 *	0.37 ns	0.91 ns
Leaf proline concentration	3.1 ns	10.4 *	4.1 *	1.6 ns	7.1 *	1.7 ns	0.01 ns
Water potential	6.8 *	9.0 *	10.8 *	0.14 ns	2.0 ns	5.0 *	0.57 ns

2.4.2 Biomass characteristics

The significant interaction between the influences of CO_2 and AM on the plant dry weight and relative growth rate (RGR) was due to a 68% and 43% decrease in mycorrhizal plants compared to non-mycorrhizal plants at 360 ppm CO_2 and a 30% and 38% increase at 5 000 ppm CO_2. The RGR of mycorrhizal plants was increased at elevated CO_2 concentrations and 0 mM NaCl compared to non-mycorrhizal plants (Figure 2.1). The plant dry weight of mycorrhizal plants increased by 50% in the presence of salinity compared to non-saline plants at 360 ppm CO_2. The significant interaction between the influences of AM and NaCl on leaf area ratio, leaf area and specific leaf area was due to a decrease in mycorrhizal plants compared to non-mycorrhizal plants at 75 mM NaCl and an increase at 0 mM NaCl (Table 2.2). The interaction between the influences of CO_2 and AM on plant moisture was due to higher moisture content of mycorrhizal than non-mycorrhizal plants at 360 ppm CO_2 and a lower moisture content of mycorrhizal plants at 5 000 ppm CO_2 (Table 2.2).

Table 2.2: Dry weight (g), moisture content (g H_2O g^{-1}DW), shoot: root ratio, relative growth rate (mg $g^{-1}d^{-1}$), leaf area (m^2), specific leaf are (m^2 kg^{-1}) and leaf area ratio (m^2 kg^{-1} plant dw), leaf, shoot and root weight ratio of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants grown in hydroponic culture with 0 and 75 mM NaCl and aeration with either ambient (360 ppm) or enriched (5 000 ppm) CO_2. Statistics as in Tabel 2.1.

characteristics	360 ppm CO_2				5000 ppm CO_2			
	0 mM NaCl		75 mM NaCl		0 mM NaCl		75 mM NaCl	
	-AM	+AM	-AM	+AM	-AM	+AM	-AM	+AM
Biomass								
Leaves	0.51 cd	0.19 a	0.58 d	0.33 b	0.4 bc	0.6 d	0.39 bc	0.53 d
Stems	0.28 c	0.05 a	0.27 c	0.14 b	0.18 b	0.33 c	0.18 b	0.26 c
Roots	0.19 c	0.07 a	0.2 c	0.15 b	0.13 b	0.21 c	0.14 b	0.23 c
Plant	0.98 c	0.31 a	1.05c	0.62 b	0.7 b	1.13 c	0.71b	1.02 c
Leaf area ratio	9. ab	41 e	13 bc	19 cd	7 a	12ab	21 d	11 ab
Leaf Area	0.008 ab	0.012 cd	0.015 cd	0.011bc	0.01 a	0.013 cd	0.02 d	0.011 bc
Specific leaf area	17 a	71 d	25 abc	35 bc	13 a	23 ab	38 c	22 ab
Shoot:root ratio	4.2 bcd	3.4 ab	4.4 bcd	3.2 a	4.5 d	4.4 cd	4.1 abcd	3.5 abc
Weight ratio Leaf	0.52 a	0.6 b	0.56 ab	0.53 a	0.55 ab	0.54 a	0.56 ab	0.52 a
Stem	0.28 c	0.17 a	0.26 bc	0.28 c	0.26 bc	0.22 b	0.25 bc	0.26 bc
Root	0.2 ab	0.23 bc	0.18 a	0.19 a	0.19 ab	0.24 c	0.2 ab	0.22 abc
Moisture content								
Plants	6.2 a	8.3 c	6.6 a	6.5 a	7.9 bc	6.5 a	6.9 ab	7.1 ab

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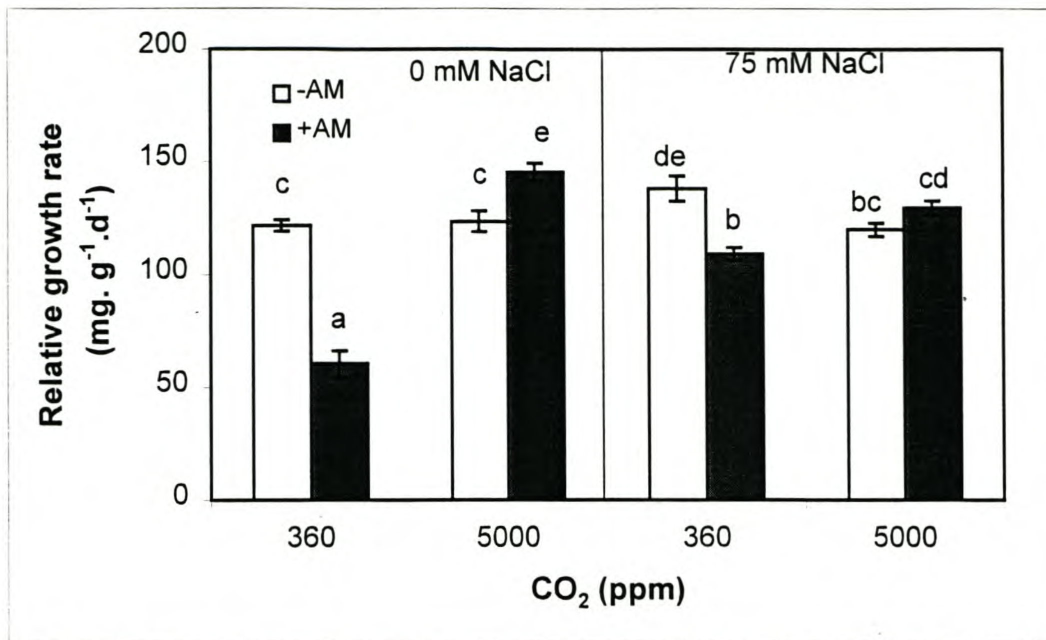

Figure 2.2: Effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 5000 ppm CO_2 on the RGR of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants (n=6). The error bars represent the SE of the means. Dissimilar letters above the bars indicate significant ($P < 0.05$) differences between means determined from analysis of variance followed by Fisher's protected LSD tests.

2.4.3 Total N

Salinity stress resulted in a decrease in the total N concentration of both mycorrhizal and non-mycorrhizal plants at 360 ppm CO_2 and mycorrhizal plants at 5 000 ppm CO_2 (Table 2.2, Table 2.3). At 5 000 ppm CO_2, salinity stress also resulted in a decreased total N concentration, but only in mycorrhizal plants. The interaction between AM and NaCl was due to a higher total N concentration at 75 mM NaCl in mycorrhizal plants than in non-mycorrhizal plants. Elevated concentrations of CO_2 decreased the plant N concentration of mycorrhizal plants at 0 mM NaCl compared to 360 ppm CO_2. The interaction between CO_2 and AM was due to a decrease in the Shoot: root N content of mycorrhizal plants compared to non-mycorrhizal plants at 360 ppm CO_2 and an increase at 5 000 ppm CO_2 (Table 2.2, Table 2.3).

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Tabel 2.3: N concentration (μmol N g^{-1} DW) of the shoot, root, plant and Shoot: root N content of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants grown in hydroponic culture with either 0 or 75 mM NaCl and aeration with either ambient (360 ppm) or enriched (5 000 ppm) CO_2 . Statistics as in Tabel 2.1.

parameters		360 ppm CO_2				5000 ppm CO_2			
		0 mM NaCl		75 mM NaCl		0 mM NaCl		75 mM NaCl	
		-AM	+AM	-AM	+AM	-AM	+AM	-AM	+AM
N	Shoot	2.49 cd	2.82 d	1.59 ab	1.22 a	2.08 bc	2.44 cd	1.53 ab	1.31 a
	Root	3.19 a	8.25 b	1.56 a	1.78 a	3.4 a	2.1 a	2.15 a	1.3 a
	Plant	2.67 c	4.03 d	1.59 ab	1.36 a	2.32 bc	2.39 bc	1.65 ab	1.30 a
S:R N content		4.09bcd	1.53 a	4.75 cd	2.55 ab	3.5 abc	5.61 d	3.14abc	3.62abcd

2.4.4 Leaf proline concentration

The leaf proline concentration was higher with salinity stress than in the absence thereof (Figure 2.3). The proline concentration was lower in mycorrhizal compared to non-mycorrhizal plants supplied with both 360 and 5 000 ppm CO_2. The interaction between CO_2 and AM was due to a smaller decrease in proline concentration due to mycorrhizal infection in plants grown at 5 000 ppm CO_2 than at 360 ppm CO_2 (Table 2.2). Elevated concentrations of CO_2 had no effect on the leaf proline concentrations of mycorrhizal or non-mycorrhizal plants. The proline concentration in the root was so low that it could not be detected by the proline determination assay.

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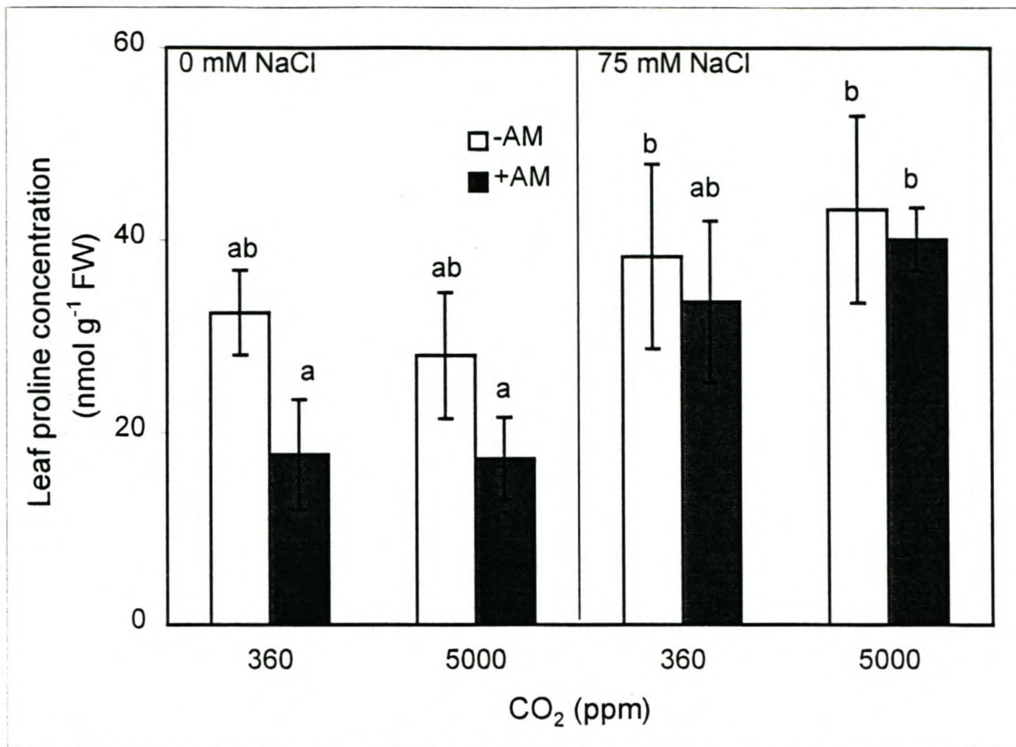

Figure 2.3: Effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 5000 ppm CO_2 on the leaf proline concentration of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants (n=6). Statistics as in Figure 2.2.

2.4.5 Water potential

Salinity stress resulted in decreased water potential of both mycorrhizal and non-mycorrhizal plants supplied with 360 ppm CO_2 compared to equivalent plants at 0 mM NaCl (Table 2.2, Figure 2.3). At 360 ppm CO_2, mycorrhizal plants had a more negative water potential compared to non-mycorrrhizal plants at both 0 and 75 mM NaCl. Elevated concentrations of CO_2 decreased the water potential of non-mycorrhizal plants compared to controls at both 0 and 75 mM NaCl.

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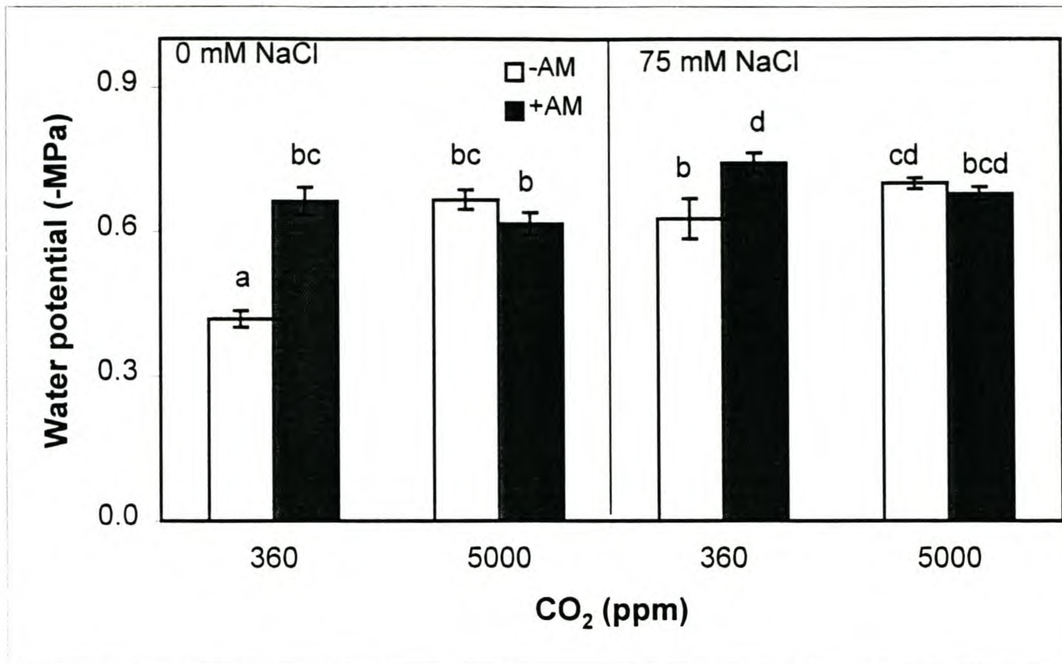

Figure 2.4: The effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 5000 ppm CO_2 on the water potential of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants (n=6). Statistics as in Figure 2.2.

2.5 Discussion

The stimulatory effect of CO_2 on arbuscular mycorrhizal infection may be due to the incorporation of DIC by PEP carboxylase that possibly provided C skeletons for the synthesis of organic and amino acids. Another possible route for the products of the incorporated DIC is the synthesis of lipids via the carboxylation of acetyl CoA to produce malonyl CoA (Sasaki *et al*, 1995), a precursor for fatty-acid biosynthesis. Since lipids form an important component of mycorrhizal structures (Wright *et al.*, 1998) the DIC incorporated into lipids may have provided additional resources for mycorrhizal growth at elevated DIC. At elevated concentrations of CO_2, the mycorrhizal infection of plants did not change in the presence of salinity stress, possibly because the anapleurotic carbon available was used for the production of compatible solutes, especially proline to assist with the alleviation of salinity stress in plants. Therefore, carbon skeletons could not be used for growth of the mycorrhizal fungus and there would not be an increase in infection. The low hyphal infection could indicate that elevated

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concentrations of CO_2 stimulated the activity of the fungus by increasing the arbuscular percentage at the cost of producing less hyphae.

Mac Donald (1982) reported an increase in infection of the non-mycorrhizal fungus *Phytophthera cryptogea* spores in chrysanthemum roots with an increase in salinity stress conditions of 75 NaCl and 150 mM $CaCl_2$. Mac Donald (1982) suggested that salt enhanced exudation of chemotactically active substances led to an increase of infective propagules in the root zone, despite a direct inhibitory effect of salinity on spore germination. It is possible that salinity had a similar effect on the mycorrhizal fungus of salinity stressed plants in the current experiment. Although the presence of salinity did not decrease the activity of the fungus as indicated by the high arbuscular percentage, the internal growth of the fungus and the ability of the fungus to store nutrients, was inhibited as indicated by the lower hyphal and vesicle infection.

The increase in mycorrhizal infection of salinity stressed plants was in contrast with findings of Duke *et al*. (1985), Poss *et al*. (1986) and Ojala *et al*. (1983), where a decrease in infection was associated with an increase in the salinity concentration in the range of between 25 and 1 00 mM NaCl. A possible reason for the difference in results of the current study compared to the studies of Duke *et al*. (1985), Poss *et al*. (1986) and Ojala *et al*. (1983) was that in the previous work, plants were grown in solid substrates like soil or sand-soil mixtures, where nutrient availability could be limited by nutrient depletion zones developing around the roots, thereby limiting nutrient uptake. Mycorrhizal hyphae in solid substrates could increase nutrient uptake. In contrast, the plants in the current experiment were grown in hydroponic culture where nutrient delivery to the root surface was ensured. The hyphae of the mycorrhizal fungus would be of little or no benefit in nutrient uptake in hydroponic culture. Eltrop and Marschner (1996)

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also reported that mycorrhizae did not improve plant growth and nutrient uptake of plants grown in semi-hydroponic culture.

Mycorrhizal plants exhibited an increase in plant dry weight and RGR due to the additional source of carbon available at elevated CO_2 and the activity of the fungus. At ambient CO_2, the decrease in the dry weight and RGR of mycorrhizal plants compared to non-mycorrhizal plants was most likely the consequence of the fungus competing with the plant for available carbon for its own growth (Wright *et al*., 1998 and Buwalda & Goh, 1982). Salinity stressed mycorrhizal plants probably had enough carbon available at elevated CO_2 to provide for its carbon requirements and to invest in thicker or denser leaves. This could dilute the effects of salinity because leaves could compartment the salt as indicated by the decrease in leaf area, SLA and LAR. The increased plant moisture content in the absence of salt and additional CO_2 could be a consequence of mycorrhizal plants containing more soluble carbohydrates for its own growth (Wright *et al*., 1998) and this could have lead to an increase in moisture content.

The decrease in total N concentration of plants in the presence of salinity could be an indication of the negative effect of salinity on the N nutrition of plants. However, this decrease in total N concentration could not be detected in the NO_3^- uptake rate of plants. Since NO_3^- uptake was measured for a limited period of a few hours, the negative effect of salinity on the total N concentration would not necessarily be reflected in the uptake results. The decrease in the shoot: root N content of mycorrhizal plants at ambient CO_2 could indicate that the products of N assimilation was diverted to the mycorrhizal fungus, while at elevated CO_2, there was an excess of N assimilation products that was translocated to the shoot.

The increase in leaf proline concentration reported in this study in the presence of salinity stress was similar to that reported by Chu *et al*. (1976), Mattioni *et al*. (1997) and Madan *et al*. (1995). The proline concentration of salinity stressed plants in this study was low compared with results reported by Mattioni *et al*. (1997) and Chu *et al*. (1976). In this investigation, plants were grown in hydroponics and would not be under severe drought stress and would therefore have lower proline concentrations than reported by other authors. The different results could also be the consequence of the extended period of exposure of plants to salinity stress. Mattioni *et al*. (1997) reported that long exposure of plants to NaCl resulted in lower proline values than observed under a shorter period (24 h) of dehydration. Sanada *et al*. (1995) and Verbruggen *et al*. (1993) suggested that although roots were the first tissues to perceive salinity stress, the levels of free proline were lower in osmotically stressed roots than in photosynthetic shoot tissue. This could possibly explain the low concentrations of proline in the root tissue.

Salinity stress possibly resulted in the increased water potential of both mycorrhizal and non-mycorrhizal plants supplied with 360 ppm CO_2. The more negative water potential of mycorrhizal plants compared to non-mycorrhizal plants at 360 ppm CO_2 may have been the consequence of mycorrhizal plants producing more soluble carbohydrates compared to non-mycorrhizal plants (Wright *et al*., 1998). The mycorrhizal plants possibly produced more soluble carbohydrates because the fungus could serve as an additional fungal sink arising through mycorrhizal colonization of the plant roots. More soluble sugars would be produced to support the growth and maintenance of the fungal symbiont. The increased water potential of non-mycorrhizal plants at elevated concentrations of CO_2 were possibly the consequence of the production of more organic and amino acids, due to the additional source of anapleurotic carbon.

Conclusion

Mycorrhizas did not improve nutrient uptake of hydroponically grown plants. The arbuscular mycorrhizal fungus competed with the host plant for carbon resources in the absence of an additional source of carbon, but the fungus could be beneficial for plant growth if the plant is provided with anapleurotic carbon.

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3 THE EFFECT OF VARIATION IN RHIZOSPHERE CO_2 CONCENTRATION AND MYCORRHIZAL INFECTION ON N NUTRITION AND ROOT RESPIRATION OF SALINITY STRESSED TOMATO PLANTS.

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Key words: respiration, Glomus mossae, salinity, rhizosphere DIC, NO_3^-

3.1 Abstract

The possibility that arbuscular mycorrhizae (AM) combined with dissolved inorganic carbon (DIC) in the root zone could alleviate the effects of salinity stress on respiration and NO_3^- uptake and metabolism was investigated. Tomato seedlings were grown in hydroponic culture (pH 5.8) with 0 and 75 mM NaCl, with or without infection by *Glomus mosseae*. The root solution was aerated with ambient CO_2 (360 ppm) or enriched CO_2 air (5 000 ppm). The CO_2 release rate of mycorrhizal and non-mycorrhizal roots was lower at elevated concentrations of CO_2 compared to plant roots at ambient CO_2, at both 0 and 75 mM NaCl. This decreased CO_2 release rate at elevated CO_2 concentrations was the result of increased incorporation of HCO_3^- by PEPc. Under conditions of salinity stress plants had a higher ratio of NO_3^-: reduced N in the xylem sap compared to plants supplied with 0 mM NaCl. Under salinity stress conditions more NO_3^- was transported in the xylem stream possibly because of the production of more organic acids instead of amino acids due to low P conditions under which the plants were grown. The NO_3^- uptake rate of plants increased at elevated concentrations of CO_2 in the absence of salinity because the HCO_3^- could be used in the production of amino acids.

3.2 Introduction

The uptake of N could be inhibited by conditions of saline stress. Hawkins and Lewis (1993), working with N concentrations of 4 mM $NaNO_3^-$ reported that NaCl did not affect NO_3^- uptake by direct competition with the NO_3^- ion. Peuke and Jeschke (1999) concluded that NO_3^- uptake was inhibited to a large extent due to an osmotic effect by NaCl and other salts. These authors investigated the effects of different salts on the high affinity transport system (HATS) with a high nitrate concentration range of 1 to 4 mM and low affinity transport system (LATS) with a low nitrate concentration range of 30 to 300 mM. Salinity stress had a negative effect on N metabolism of salinity stressed plants by causing NO_3^- reduction and assimilation to be shifted from the shoot to the root, with associated increased energetic costs (Cramer *et al*., 1995). Lower concentrations of NO_3^- relative to reduced N in the xylem sap of salinity stressed plants was reported as a consequence of lower rates of NO_3^- uptake and limited NO_3^- loading into the xylem (Cramer *et al.*, 1995). Furthermore, the NO_3^- concentration in the stem and leaves as well as the NR activity of the leaves were decreased in salinity stressed plants.

The growth of salinity stressed plants could be improved by the addition of DIC (CO_2 and HCO_3^-) to the root medium (Cramer and Lips, 1995). The enzyme responsible for the assimilation of inorganic carbon is phosphoenolpyruvate carboxylase (PEPc) and its functions include replenishment of the TCA cycle intermediates, refixation of respired CO_2, pH stat functioning and participation in N assimilation (Vuorinen and Kaiser, 1997). The addition of DIC in the rhizosphere resulted in an increase in NO_3^- uptake by salinity stressed plants (Cramer *et al.*, 1995). Furthermore, labelling experiments with $H^{14}CO_3^-$ supplied to salinised plants indicated that the products of $DI^{14}C$ incorporation were preferentially diverted into amino acid synthesis and transported in the xylem stream to the shoot, whereas non-salinised plants accumulated more ^{14}C as organic acids (Cramer and Lips, 1995). These authors

concluded that DIC supplied to the root zone could increase the possible uptake of NO_3^- and provide anapleurotic carbon for amino acid synthesis in roots of plants that were forced by salinity to reduce NO_3^- in the root.

Arbuscular mycorrhizas (AM) are obligate symbionts and require organic compounds from their hosts. Buwalda and Goh (1982) reported that AM depleted the host C supplies to such an extent that growth depressions occurred. Jakobsen and Rosendahl (1990) reported that carbon allocation to the external hyphae was about 4% of the photosynthetic assimilated ^{14}C and it was estimated that the fungal biomass of the VA mycorrhizas and its respiration consumed 20% of the photo-assimilated ^{14}C. It was suggested that the C allocated to arbuscular mycorrhizal fungi could be used for the production of lipids and other internal fungal structures (Douds *et al.*, 1988 and Buwalda & Goh, 1982). Wright *et al.* (1998) reported a significantly higher C gain by mycorrhizal plants compared to non-mycorrhizal plants that could have been the result of increased sink strength provided by the mycorrhizal fungus. Wright *et al.* (1998) suggested that the increased pools of C in mycorrhizal roots were sufficient both to support root and fungal growth, maintenance and storage.

It is well known that mycorrhizas can improve the P uptake of plants, but it has also been shown that mycorrhizas were able to assist the plant with N uptake and influence N metabolism (Azcon *et al.*, 1992). Johansen *et al.* (1993, 1994) reported that mycorrhizal hyphae was able to transport N as $^{15}NO_3^-$ and $^{15}NH_4^+$ from a hyphal compartment over several centimetres to the roots of cucumber plants. Although these authors were able to show N transported to the plant root, the mycorrhizas did not always improve plant growth due to increased hyphal N uptake (Johansen *et al.*, 1993). Johansen *et al.* (1996) found concentrations of asparagine, constituting 70% of the free amino acid pool in the external mycorrhizal hyphae and concluded that the

external hyphae of the AM fungus was capable of taking up NH_4^+ and NO_3^- and assimilating it into the pool of free amino acids. Azcon *et al.* (1992) reported higher nitrate reductase (NR) and glutamine synthase (GS) activities in plants colonised by mycorrhizae and concluded that the increased enzyme activities were due to the presence of the fungus.

The process of respiration could be affected by factors such as salinity stress, AM and DIC. Salinity stress influenced respiration by increasing the maintenance respiration of salinity stressed plants as reported by Schwarz and Gale, 1981 and Penning De Vries, 1975. However, Blacquire and Lambers, 1981 and Taleisnik, 1987 considered an increase in maintenance respiration of minor importance during salinity stress. The influence of mycorrhizae was reported as increasing root respiration (measured as CO_2 release) that was attributed to additional fungal respiration (Baas *et al.*, 1989). Hawkins *et al.* (1999) reported an increase in the root respiration of mycorrhizal plants measured as CO_2 release rate, but no increase in O_2 consumption was reported. DIC supplied to the root zone influenced root respiration by a 36% increase in the root O_2 consumption when a concentration of 2 000 ppm CO_2 was applied, while the CO_2 efflux from the roots decreased (Van der Westhuizen and Cramer, 1998). This increase in root O_2 consumption was associated with energy requirements for increased NO_3^- uptake or for effects of DIC incorporation on TCA cycle activity and the decrease in CO_2 efflux was caused by the incorporation of DIC into organic acids through root PEPc activity.

In this investigation the role of elevated DIC supplied to the root zone and AM in alleviating salinity stress in hydroponically grown tomato plants was investigated. The effect of DIC, salinity and AM on the uptake and assimilation of NO_3^- and respiration was determined.

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3.3 Materials and methods

3.3.1 Growth conditions

Seeds of *Lycopersicon esculentum* L. Mill F114 were germinated in pots (12.5 cm in diameter) of sterilised sand mixed with 10 g of live mycorrhizal inoculum of *Glomus mosseae* (supplied by Agricultural Genetics Co. LTD, UK). The composition of the inoculum and the treatment of the sand and inoculum were similar to that described by Lintnaar *et al.* (Chapter 2.3.1) Seedlings were watered to field capacity with Long Ashton nutrient solution (Hewitt, 1966) modified to contain 50 μM Na-phosphate and 0.1mM MES. $NaNO_3$ was used as N source and the pH was maintained at 5.8 by adjusting with NaOH and HCl daily. The non-mycorrhizal plants received benlate as described by Lintnaar *et al.*, (Chapter 2.3.1). The plants received 75 mM NaCl as salinity treatment supplied over a period of 2 d. The plants were grown in a greenhouse between June and August with a midday irradiance and 550 and 650 μmol m^{-2} $s,^{-1}$ an average day / night temperature of 23 / 15°C and relative humidity of 35 / 70%.

After 5 weeks in the sand, the inoculated seedlings were transferred into 22 l hydroponic tanks after carefully rinsing the roots with deionised water. The nutrient solution was replaced every 5 d and the pH of the medium was maintained at 5.8 by adjusting with NaOH and HCl daily. The CO_2 was supplied from a cylinder of industrial grade CO_2 and mixed with compressed air to obtain the appropriate CO_2 concentration. The composition and circulation of the nutrient medium was as described by Lintnaar *et al.*, (Chapter 2.3.1). The CO_2 concentration was monitored continuously using an ADC Model 225 MK3 infra red gas analyzer (Analytical Development Corporation, Hoddesdon, UK) set up in absolute mode with a resolution of between 10 and 20 ppm CO_2. The CO_2 concentrations were maintained at either 360 or 5 000 ppm.

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3.3.2 Experimental design and statistical analysis

The experimental design comprised of 3 factors, with two levels each. The factors were AM inoculation (live or no inoculum), salinity (0 and 75 mM NaCl) and CO_2 (360 and 5000 ppm CO_2). Each treatment was replicated 6 times however, all the plants of one treatment were grown in one tank. The effects of the treatment were tested using analysis of variance (ANOVA) and a LSD test was used Statgraphics Vers.7.0. Prior to analysis of variance, percentage data were arcsine transformed (Zar, 1981).

3.3.3 Mycorrhizal infection

Root segments were excised and cleared with KOH, acidified with HCl and stained with Analine blue and the extent of infection determined as described by Lintnaar *et al.*, (Chapter 2.3.3).

3.3.4 Respiration measurement

Six plants of each treatment were grown in hydroponic culture for 7 days before being transferred to cuvettes for respiration measurements. The cuvettes had three ports, one connected to the air source, one for the addition of nutrient solution and one for sampling the gas (van der Westhuizen and Cramer, 1998). The cuvettes contained 298 ml stirred Long Ashton nutrient solution 2.5 mM MES (pH 5.8) and 4 mM $NaNO_3^-$. The air temperature was controlled at 20^0C and a continuous light source of 350 μmol m^{-2} s^{-1} was supplied. Air (360 ppm CO_2) was supplied through precision needle valves to each plant root at *c* 140 ml min^{-1}. After a period of acclimatization (12 h), the roots were aerated with either 360 or 2 000 ppm CO_2. The CO_2 concentration of the infrared gas analyser was calibrated and kept at 2000 ppm CO_2 because the accuracy of the measurement decreased above 2 000 ppm CO_2. Calibration was performed by mixing pure CO_2 with N_2 in a gas mixing syringe (Li-Cor Inc. Model 6 000-

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01, Lincoln, NE). The CO_2 was supplied from a cylinder of industrial grade CO_2 mixed with compressed air to obtain the appropriate CO_2 concentration. The CO_2 flux from the root was measured with an ADC-225-MK3 infrared gas analyser (Analytical Development Corporation, Hoddesdon, UK).

Root O_2 consumption was measured with polarographic O_2 electrodes (Yellow-Springs Instrument Co. Inc., Yellow-Springs, Ohio) immersed in the nutrient solution through a port in each cuvette. Immediately prior to each O_2 measurement, the air supply to the root solution of each plant was discontinued, gas bubbles flushed from the cuvettes and depletion of O_2 measured over 20 to 30 min. After the measurements, the plants were harvested, divided into shoot and root components, dried in the oven at 80°C for 48 h, reweighed and the respiration rate expressed on the basis of root dry mass.

3.3.5 Plant harvest and chemical analysis

Plants were harvested 10 d after transfer into hydroponic culture. The plants were divided into leaf, stem and root components and weighed to determine the fresh weight. The roots were carefully blotted dry and a piece of the root was cut off, weighed and stored in a vial with 50% (v/v) ethanol for estimation of mycorrhizal infection. The components were dried in an oven at 80°C for *c* 48 h and weighed to determine the dry weight.

Xylem sap (*c*. 50 to 150 µl) was collected between 9:00 and 12:00 in the morning with a pressure chamber (PMS instruments Co, Oregan USA) at an applied pressure of 1 MPa for 5 to10 min and temporarily stored on ice and subsequently at –18°C. The xylem sap samples were analysed for NO_3^- using the copper cadmium method (Nydahl, 1976) modified for assay

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of small volumes. The reduced-N concentration was determined using the ninhydrin assay of Rosen (1957) with glycine as the standard.

3.3.6 NO_3^- uptake

Five plants of each treatment were transferred to 300 ml cuvettes containing fresh Long Ashton nutrient solution and 2.5 mM MES (pH 5.8) and 0.2 mM $NaNO_3$. The solutions were aerated with either 360 or 5 000 ppm CO_2. The plants were equilibrated for 12 h before the beginning of the experiment at a temperature of 20°C and an irradiance of 450 μmol m^{-2} s^{-1}. NO_3^- uptake was measured as depletion from an initial concentration of 1 mM NO_3^-. Samples of 1 ml were taken at the beginning of the experiment and at 2 h intervals for 8 h thereafter. At the end of the experiment, the volume of the solution was determined and plants divided into shoot and root components, the roots rinsed in distilled water, blotted dry and weighed. The concentration of NO_3^- in the nutrient solution was measured by using the Cu-Cd method (Nydahl, 1976) modified for assay of small volumes.

3.3.7 Nitrate reductase activity

Six plants of each treatment were harvested and root and shoot samples were quenched in liquid N_2 before storage at -80°C. Leaf and root soluble proteins were extracted by homogenising the frozen material in a precooled mortar in a ratio of 1 g material to 4 ml of ice-cold extraction buffer. The extraction medium for NR (NADH: nitrate reductase, EC 1.6.6.1) contained 200 mM Tris HCl buffer (pH 7.8), 2 mM EDTA, 3 mM dithiothreitol, 2% (w/v) casein, 10% (v/v) glycerol and 0.1g of polyvinylpyrrolidone per sample (Cramer *et al.*, 1999). All extracts were centrifuged at 12 000 g in an IEC Model B-20 (Needham HTS, Mass. USA) refrigerated centrifuge at 4°C for 10 min and the supernatant was used for the assays. The reaction mixture (200μl) for NR contained 30 mM phosphate buffer, 50 mM Tris / HCl buffer

(pH 7.5), 25 mM KNO_3 and 0.4 mM NADH. The reaction was initiated by the addition of 200 µl of enzyme extract and incubated at 30°C for 10 min. The reaction was stopped by the addition of the reagents for NO_2^- determination (Snell and Snell, 1949). The samples were centrifuged for 30 s and the absorbence was measured 540 nm. NR activity was calculated from the amount of NO_2^- accumulated.

3.4 Results

3.4.1 Mycorrhizal infection

The percentages of hyphal and total infection of non-mycorrhizal plants were very low compared to mycorrhizal plants (Table 3.1) and therefore non-mycorrhizal plants will not be further discussed. Component infections of mycorrhizal plants were dominated by hyphae; arbuscular and vesicle infection made up less than 5% of the total infection (Table 3.1). Plants supplied with 75 mM NaCl had an increased hyphal infection at both 360 ppm and 5 000 ppm CO_2, compared to controls (Table 3.1).

Table 3.1:The percentages of arbuscules (q), hyphae (u), vesicles (r) and total infection (100*(q+r+u)/G) per root length of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants grown in hydroponic culture with 0 and 75 mM NaCl and aeration of the root solution with either ambient (360 ppm) or enriched (5 000 ppm) CO_2. The means are followed by letters indicating whether salinity and CO_2 had a significant influence ($P < 0.05$, Fisher`s protected LSD) (n=6).

parameters	360 ppm CO_2				5 000 ppm CO_2			
	0 mM NaCl		75 mM NaCl		0 mM NaCl		75 mM NaCl	
	-AM	+AM	-AM	+AM	-AM	+AM	-AM	+AM
% Arbuscules	0.4a	3.9b	0.2a	1.4a	0a	1.4a	0a	2.3ab
% Vesicles	0.2a	1.5a	0.2a	2.4ab	0.2a	0.2 a	0a	2.6ab
% Hyphae	5.7b	19c	4.9b	25 d	1.9a	18c	5.6b	29de
% Total infection	6.2b	25d	5.3b	28 d	2.1a	20c	5.6b	34e

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Table 3.2: Sources of variation expressed as F-ratios for mycorrhizal infection, respiration measurements and NO_3^-: Reduced N ratio, NO_3^- uptake, shoot and root NR activities and the percentage NR distribution of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants grown in hydroponic culture with either 0 or 75 mM NaCl and aeration of the root solution with either ambient (360 ppm) or enriched (5 000 ppm) CO_2.

	Sources of variation						
	Factors			Interactions			
	CO_2	NaCl	AM	CO_2xNaCl	CO_2 x AM	AM xNaCl	CO_2xNaClxAM
Mycorrhizal infection							
Arbuscules	15.54 *	12.72*	25.51*	0.44 ns	0.40 ns	37.69*	9.97*
Vesicles	4.15 *	1.76 ns	18.79*	2.09 ns	16.0*	5.49 *	0.28 ns
Hyphae	5.85 *	7.92 *	53.37 *	0.36 ns	36.72 *	53.43 *	41.48*
Total infection	2.85 ns	10.20 *	39.44 *	0.11 ns	2.28 ns	33.25 *	8.04 *
Respiration							
O_2 consumption	0.12 ns	1.00 ns	3.16 ns	2.52 ns	2.28 ns	9.02 *	2.82 ns
CO_2 release	347.80 *	2.25 ns	0.03 ns	30.53 *	1.56 ns	4.50 *	1.76 ns
RQ value	223.37 *	0.98 ns	0.01 ns	10.17 *	0.42 ns	0.60 ns	0.08 ns
Nitrogen data							
NO_3^-: RN ratio	2.43 ns	16.70 *	0.54 ns	0.20 ns	1.79 ns	0.09 ns	1.75 ns
NO_3^- uptake	1.17 ns	0.18 ns	3.38 ns	1.91 ns	0.57 ns	0.03 ns	1.71 ns
NR activity Leaf	24.52 *	7.96 *	0 ns	1.22 ns	3.96 ns	0.59 ns	2.15 ns
Root	5.78 *	0.29 ns	0.15 ns	0.29 ns	1.39 ns	2.53 ns	0.67 ns
NR distribution Leaf	10.88 *	27.9 *	0.025 ns	1.76 ns	1.05 ns	5.63 *	2.01 ns
Root	10.58 *	1.47 ns	0.40 ns	1.03 ns	2.74 ns	7.1 *	3.62 ns

Mycorrhizal infection and NR distribution data was arcsine transformed. The table represents the results of a 3-way ANOVA; df:, total =40; * $P \leq 0.05$, ns = not significant.

3.4.2 Respiration measurements

The interaction between AM and NaCl for O_2 consumption was due to an increase in O_2 consumption of mycorrhizal roots compared to non-mycorrhizal roots at 0 mM NaCl and a decrease at 75 mM NaCl (Table 3.2, Figure 3.1). Plants supplied with both 0 and 75 mM NaCl, had no significant differences between the O_2 consumption rate of non-mycorrhizal and mycorrhizal roots at 360 and 2 000 ppm CO_2. Elevated concentrations of CO_2 resulted in a decrease in the CO_2 release rates and Rq's of roots at both 0 and 75 mM NaCl (Figure 3.2). The interaction between CO_2 and NaCl was due to a larger decrease in the CO_2 release rate and Rq due to elevated CO_2 in plant roots supplied with 0 mM NaCl than in plants supplied with 75 mM NaCl. Mycorrhizal and non-mycorrhizal roots supplied with elevated CO_2 concentrations had a decreased CO_2 release rate at 0 mm NaCl compared to 75 mM NaCl.

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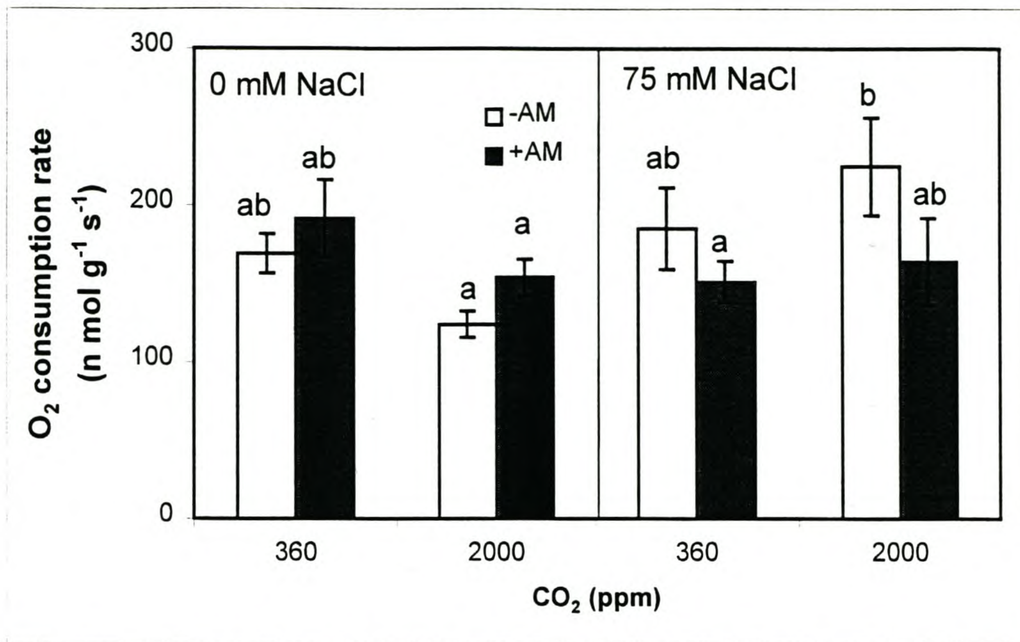

Figure 3.1: The effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 2000 ppm CO_2 on the O_2 consumption rate of mycorrhizal plants (+AM) and non-mycorrhizal (-AM) tomato plants (n=6). The error bars represent the SE of the means. Dissimilar letters above the bars indicate significant ($P < 0.05$) differences between means determined from analysis of variance followed by Fisher's protected LSD test.

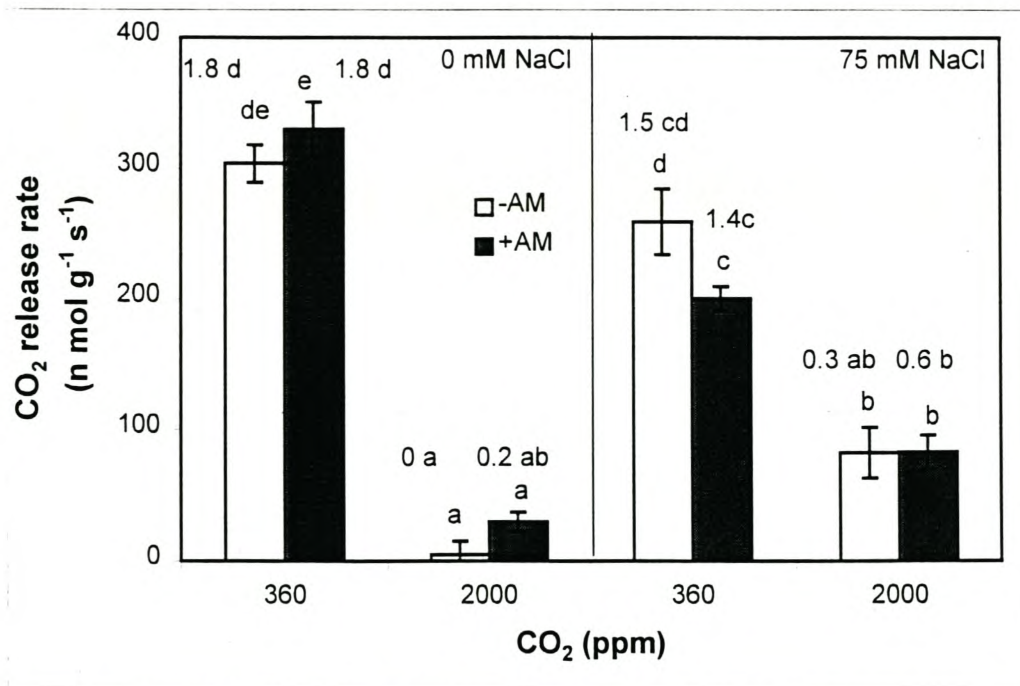

Figure 3.2: The effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 2000 ppm CO_2 on the CO_2 release and Rq of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants (n=6). Statistics as in Figure 3.1. CO_2 release and Rq were tested separately.

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3.4.3 Xylem NO_3^-: Reduced N ratio

Salinity stress resulted in an increase in the NO_3^-: reduced N ratio in both mycorrhizal and non-mycorrhizal plants supplied with 5 000 ppm CO_2, compared to controls (Table 3.2, Figure 3.4). The NO_3^-: reduced N ratio of plants with 0 or 75 mM NaCl was not altered by CO_2 concentrations.

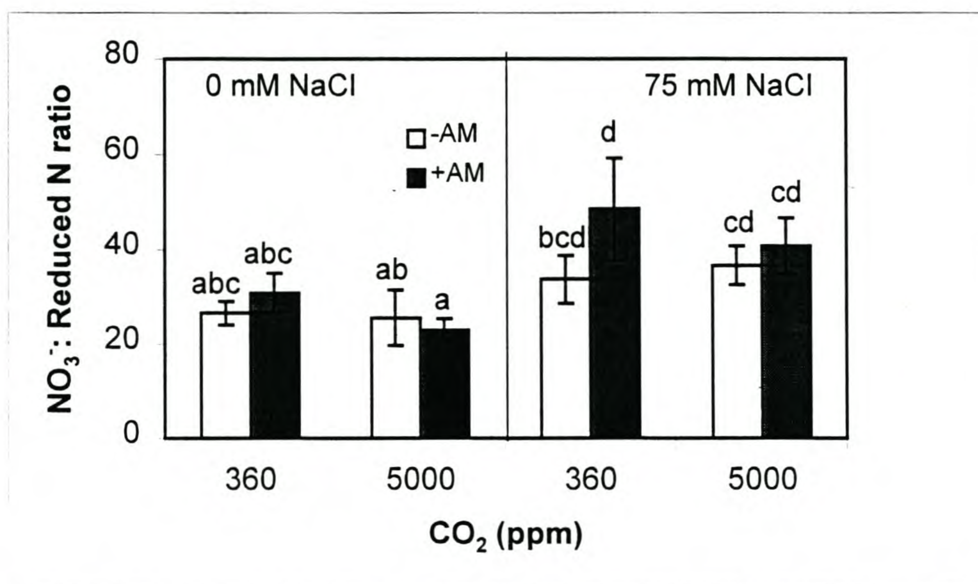

Figure 3.3: The effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 5000 ppm CO_2 on the NO_3^-: reduced N ratio of mycorrhizal plants (+AM) and non-mycorrhizal (-AM) tomato plants. Plants were grown in hydroponics and supplied with NO_3^- nutrition. Statistics as in Figure 3.2 (n=6).

3.4.4 NO_3^- uptake rate

No significant influence of CO_2, NaCl or AM on NO_3^- uptake was found in the analysis of variance. However, at 0 mM NaCl the NO_3^- uptake rate of mycorrhizal and non-mycorrhizal plants supplied with 5 000 ppm CO_2 was increased compared to equivalent plants at 360 ppm CO_2 (Figure 3.4). At 75 mM NaCl, plants supplied with 5 000 ppm CO_2 had a decreased NO_3^- uptake rate compared to plants at 0 mM NaCl (Figure 3.4). The NO_3^- uptake rate of plants supplied with 75 mM NaCl was not changed with CO_2 concentration.

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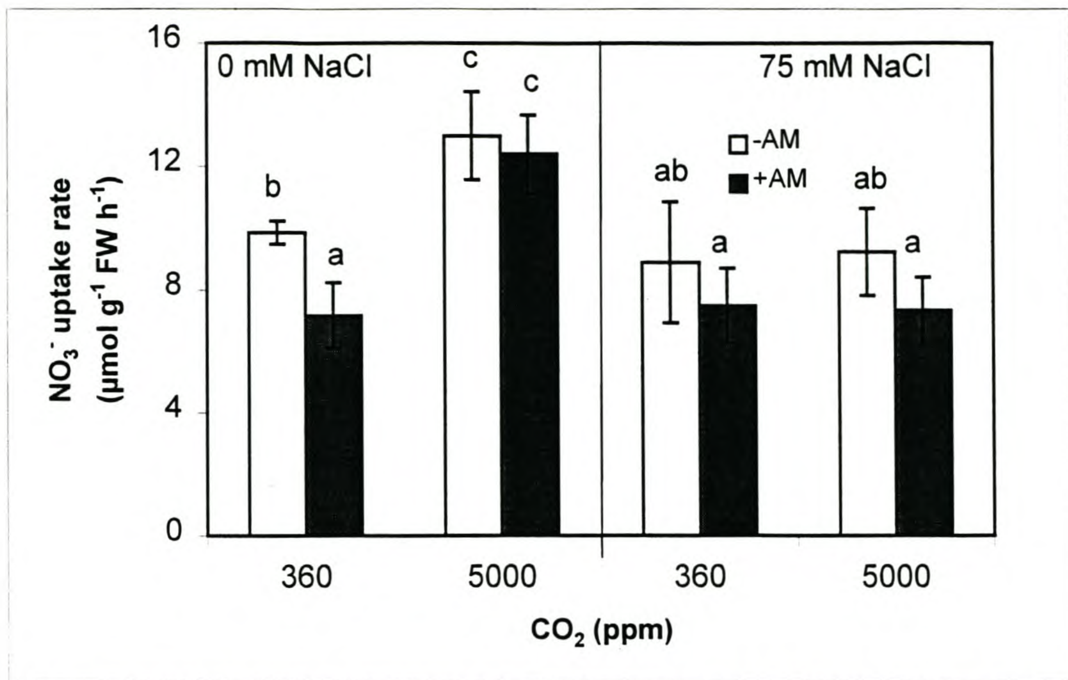

Figure 3.4: The effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 5000 ppm CO_2 on the NO_3^- uptake rate of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants. The letters indicate whether the treatments had a significant influence ($P < 0.05$) on the NO_3^- uptake rate of plants. Bars indicate the SE of the mean (n=6).

3.4.5 Nitrate reductase activity

Elevated concentrations of CO_2 increased the leaf NR activity of both mycorrhizal and non-mycorrhizal plants at both 0 and 75 mM NaCl (Table 3.2, Figure 3.5). Salinity stress caused an increase in the leaf NR activities of mycorrhizal and non-mycorrhizal plants supplied with 5 000 ppm CO_2 and non-mycorrhizal plants at 360 ppm CO_2 (Table 3.2, Figure 3.5). At 0 mM NaCl, elevated concentrations of CO_2 decreased the root NR activity of mycorrhizal plants compared to control plants (Figure 3.7). The negative effect of elevated CO_2 was the consequence of the very significant decrease in root NR activity of mycorrhizal plants supplied with 5 000 ppm.

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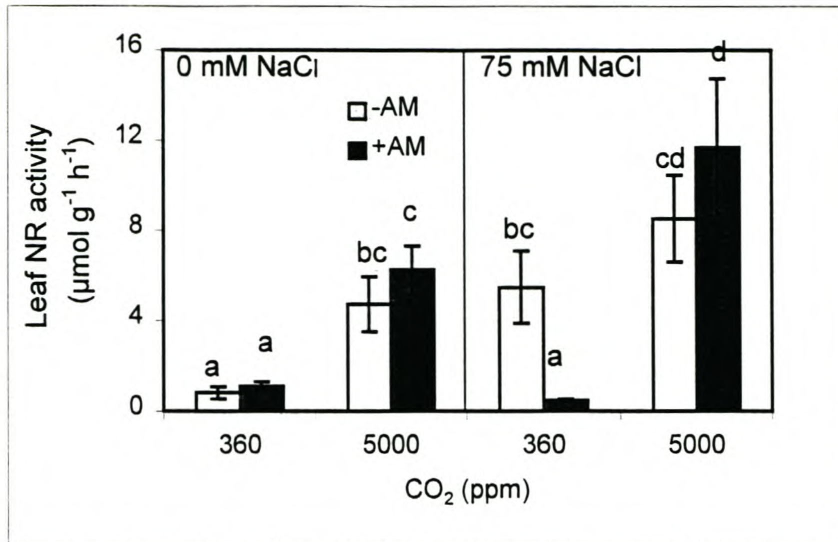

Figure 3.5: The effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 5000 ppm CO_2 on the leaf NR activities of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants (n=6). Statistics as in Figure 3.4.

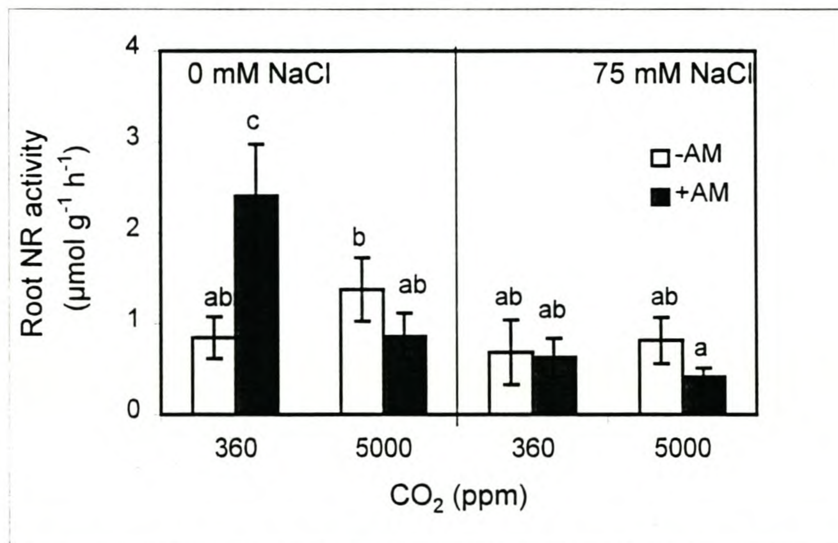

Figure 3.6: The effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 5000 ppm CO_2 on the root NR activity of mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants (n=6). Statistics as in Figure 3.4.

The mycorrhizal plants had a higher proportion of leaf NR at elevated concentrations of CO_2 compared to controls at both 0 and 75 mM NaCl (Table 3.2, Figure 3.7). The presence of salinity stress resulted in an increased proportion of leaf NR in non-mycorrhizal plants compared to controls at both 360 and 5 000 ppm CO_2. The proportion of root NR of

mycorrhizal plants were less at elevated concentrations of CO_2 compared to controls, at both 0 and 75 mM NaCl (Table 3.2).

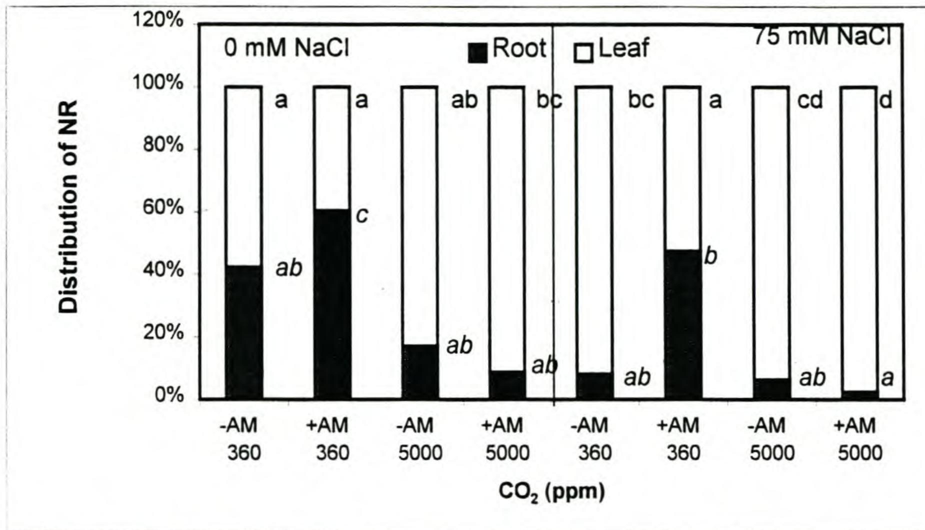

Figure 3.7. The effect of 0 and 75 mM NaCl and aeration with either 360 ppm or 5000 ppm CO_2 on the shoot and root NR distribution in mycorrhizal (+AM) and non-mycorrhizal (-AM) tomato plants (n=6). Statistics as in Figure 3.4.

3.5 Discussion

In this investigation, the mycorrhizal infection patterns were different because the arbuscular component was lower in favour of a higher hyphal component. This could be an indication that the mycorrhizal symbiosis was not very active. Blee and Anderson (1998) suggested that arbuscules are the site for nutrient exchange between the fungus and the plant. The mycorrhizas possibly had a limited impact on the physiology of the host plant, because of the lower arbuscule percentage. Salinity stress stimulated hyphal infection and possibly had the same effect as discussed in Chapter 2.5 (Lintnaar *et al.,* Chapter 2).

The lack of differences in the O_2 consumption rate of non-mycorrhizal roots supplied with elevated CO_2 concentrations and 0 mM salt compared to equivalent plant roots at ambient CO_2, was in contrast with results of van der Westhuizen and Cramer (1998) who found a 36% increase in root O_2 consumption with an increase in the rhizosphere CO_2 concentration. In their

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study, plants were supplied with ambient CO_2 only for a few hours, but in the current investigation plants were supplied with elevated CO_2 for the entire growth period. The increased O_2 consumption of roots supplied with elevated CO_2 could be a short term response that would only occur if plants were supplied with elevated CO_2 for a relatively short period. The similar O_2 consumption of mycorrhizal and non-mycorrhizal plant roots supplied with ambient concentrations of CO_2 was in agreement with Hawkins *et al.* (1999), who attributed their result to the lack of improved nutrient uptake by the mycorrhizal roots. Johansen *et al.* (1994) reported that mycorrhizal hyphae were able to transport N from the soil to the host plant. However, in the present study the relatively lower colonisation of the fungus and low symbiotic activity, may also explain the lack of change in O_2 consumption rates of plant roots supplied with ambient and elevated CO_2 concentrations.

The lower CO_2 release rates and Rq values for both mycorrhizal and non-mycorrhizal plant roots supplied with elevated concentrations of CO_2 compared to roots supplied with ambient CO_2 were likely the result of the increased rate of incorporation of HCO_3^- by PEPc (Van der Westhuizen and Cramer, 1998). The CO_2 release rate and Rq values both decreased at elevated CO_2 concentrations without an increase in O_2 consumption. The interaction between salinity and CO_2 significantly changed the response of PEPc incorporation in plants supplied with elevated and ambient CO_2. The changes in Rq values indicated that in the presence of salinity the incorporation of HCO_3^- via PEPc increased in plants supplied with ambient CO_2, but the incorporation of HCO_3^- decreased at elevated concentrations of CO_2, assuming that the changes in Rq were due to incorporation of HCO_3^- by PEPc. The lower CO_2 release rates of plant roots supplied with elevated concentrations of CO_2 and 0 mM NaCl compared to equivalent plants at 75 mM NaCl could be associated with increased carbon utilisation, possibly for NO_3^- uptake.

The concentrations of xylem NO_3^- and xylem reduced N may vary due to the flow rate of the xylem sap (Cramer *et al.*, 1995) and were expressed as a ratio to control this variation of flux in the xylem sap. The higher ratio of NO_3^- transported in the xylem sap under conditions of salinity stress and elevated concentrations of CO_2 was in contrast with findings by Cramer and Lips (1995). These authors reported that a higher ratio of amino acids to NO_3^- was transported in the xylem sap under conditions of elevated CO_2 concentrations and salinity stress. This was attributed to salinity causing a shift in NO_3^- assimilation from shoot to root. In addition, elevated DIC supplied to the rhizosphere made anapleurotic carbon available for amino acid synthesis. A possible reason for the higher NO_3^- ratio in the xylem sap of salinity stressed plants could be the consequence of plants growing under concentrations of low P. Carbon skeletons, derived from the increased rate of incorporation of HCO_3^- by PEPc, could have been used for the production of organic acids to increase the availability of P under conditions of P limitation (Johnson *et al.*, 1996). Cramer *et al.* (1995) conducted their experiments under relatively high P concentrations (2 mM) while in the present investigation, plants were grown under P limited conditions (50 μM).

The increased NO_3^- uptake rate of plants supplied with elevated concentrations of CO_2 in the absence of salinity stress, was also reported by Cramer and Lips (1995). The conversion of CO_2 to HCO_3^- and subsequent incorporation of HCO_3^- through the activity of PEPc (Cramer *et al.*, 1996) could provide carbon that could be used with NO_3^- for the production of amino acids. The decrease in NO_3^- uptake rate of plants supplied with elevated concentrations of CO_2 and salinity was in contrast with results by Cramer and Lips (1995), where an increase in NO_3^- uptake was reported at elevated concentrations of CO_2 and 100 mM NaCl. The lack of difference in the NO_3^- uptake rate of plants supplied with both ambient and elevated concentrations of CO_2 and 75 mM NaCl could be the consequence of plants growing under low

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P conditions. Under conditions of salinity stress, NO_3^- uptake was probably not promoted because the C-skeletons were used for organic acid synthesis and diverted carbon away from the synthesis of amino acids.

The increase in root NR activity at ambient concentrations of CO_2 compared to plants supplied with elevated CO_2 was also reported by Cramer and Lips (1995). The relatively low root NR activity of plants supplied with salinity compared to plants grown without salinity (Figure 3.6) could possibly be due to the production of less amino acids in favour of increased organic acid production due to the low P (50 μM) conditions. The increased leaf NR activity of plants supplied with elevated concentrations of CO_2 at 0 and 75 mM NaCl was also reported by Cramer and Lips (1995). The relatively higher leaf NR activities of plants supplied with 75 mM NaCl compared to equivalent plants at 0 mM NaCl could be the consequence of more NO_3^- being transported in the xylem as indicated by the NO_3^-: reduced N ratio. This could increase NO_3^- availability for NO_3^- reduction in the shoots. The increased leaf NR activity of plants supplied with elevated concentrations of CO_2 and 0 mM NaCl compared to equivalent plants supplied with ambient CO_2 concentrations could be correlated with the increased NO_3^- uptake rate of plants supplied with elevated CO_2 and 0 mM NaCl.

The proportion of root NR followed the same trend as leaf NR with an increase in plants supplied with elevated concentrations of CO_2, while the presence of salinity decreased the proportion of NR present in the root. AM modified the distribution of root NR by increasing the proportion of NR in mycorrhizal plants supplied with ambient concentrations of CO_2. The same pattern was followed at 0 and 75 mM NaCl, but only differed in scale. The higher proportion of NR in mycorrhizal plants supplied with 360 ppm CO_2 and 75 mM NaCl was possibly due to the increased infection of mycorrhizal plants supplied with 75 mM NaCl.

Conclusion

The incorporation of HCO_3^- via PEPc was increased by the presence of salinity in plants supplied with ambient CO_2 concentrations. Low concentrations of P was possibly responsible for the change in composition of DIC assimilation products from amino acids to organic acids in plants grown with elevated CO_2 concentrations and salinity stress conditions. Elevated concentrations of CO_2 increased the leaf NR activity of plants.

Acknowledgements:

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4 GENERAL CONCLUSION

For this study, the physiological effects of arbuscular mycorrhizas and elevated concentrations of DIC supplied to the root zone were evaluated to determine if it could alleviate salinity stress on hydroponically grown tomato plants. It was hypothesised that elevated concentrations of DIC supplied to the root zone would be able to improve plant growth and alleviate salinity stress due to the provision of carbon for the synthesis of organic acids and amino acids and also to improve N-nutrition of plants. It was also hypothesised that elevated concentrations of DIC in the root zone could stimulate the growth of the AM fungus. The AM fungus in turn could improve plant growth and alleviate salinity stress possibly by improved nutrient uptake.

Different seasons influenced the percentages as well as the patterns of infection of mycorrhizal plants grown between June and August (winter) and November and December (summer). The percentages of arbuscules were 90% lower and total infection was 25% lower in winter (Table 3.1) compared to infection data of mycorrhizal plants grown in summer (Table 2.1). In mycorrhizal plants grown in winter, hyphal infection was the predominant component (83% of mycorrhizal infection) and this indicated that mycorrhizal infection was probably in its developmental stages. During the early stages of colonisation, fungal infection begins with the formation of an appressorium that penetrates the epidermis cells and is followed by the formation of hyphae that could branch into the middle and inner cortex of the root (Smith and Read, 1997). The low arbuscular presence further supports the argument that the plants were still in the development stages of mycorrhizal growth. During the final stages of colonisation, arbuscules are formed that could provide a considerable increase in surface area of contact between fungus and the plant. Blee and Anderson (1998) suggested that arbuscules are the site for nutrient exchange between the fungus and the plant and where arbuscules were few, it could be argued that the symbiosis would be less active or under-developed. Furthermore, a study of

arbuscular mycorrhizal spores revealed that mycorrhizal infection increased in summer and was followed by a drop in infection in autumn and an increase during spring (Mason 1964, Hayman 1970 and Sutton & Barron, 1971). This change in mycorrhizal infection was linked to the growth of the host plant and possibly due to the availability of carbohydrates for fungal growth (Graham *et al*., 1981). A decreased arbuscular mycorrhizal infection would have limited the impact of infection on the physiology of the host plant.

In the present study, hydroponically grown AM plants did not improve NO_3^- uptake, despite evidence by Johansen *et al*. (1994) that AM hyphae can transport N over distances of several centimetres to the root. This may have been a consequence of the growth of arbuscular mycorrhizae in hydroponic culture. Hawkins *et al*. (1999) reported that arbuscular mycorrhizal infection did not increase the concentrations of N and P in hydroponically grown wheat plants. Eltrop and Marschner (1996) also reported that nutrient uptake of Norway spruce seedlings was not increased by the presence of ectomycorrhizal fungi in semi-hydroponic culture. A limitation for mycorrhizal growth in hydroponics is that the nutrients that are relatively immobile in the soil are freely available and the benefit that mycorrhizal hyphae normally have assisting with nutrient uptake would be neutralised (Hawkins and George, 1997). However, soil-based studies are limited in that rates of nutrient supply at the root surface cannot be manipulated easily and the root and fungus cannot be harvested without significant damage. Such roots are often unsuitable for studying nutrient metabolism of roots (Hawkins and George, 1997).

Cramer and Lips (1995) that did previous work on the effect of DIC on N nutrition reported that increased NO_3^- uptake and elevated concentrations of DIC supplied to the root zone were able to improve plant growth in salinity stressed plants. In this investigation, the increase in dry

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weight and RGR of mycorrhizal plants at elevated concentrations of CO_2 was associated with increased NO_3^- uptake rate of plants, but not in the presence of salinity as was found by Cramer and Lips (1995). NO_3^- uptake was not promoted because the C skeletons were used for the production of organic acids that diverted carbon away from amino acid synthesis. Under salinity stress conditions, mycorrhizal plants at elevated CO_2 showed an increased plant dry weight and RGR, even though the NO_3^- uptake rate did not increase. This was due to elevated concentrations of DIC providing C skeletons for plant growth as well as for arbuscular mycorrhizal development.

The decrease in the total N concentration of plants supplied with salinity was not reflected in the NO_3^- uptake rate. This difference in results could be because the NO_3^- uptake rate of plants were measured over a period of a few hours, while the total N concentration was measured for the entire growth period. Therefore, the decrease in total N concentration caused by salinity stress was a long term effect that could not be detected after a period of a few hours. Furthermore, the NO_3^- uptake rate only gives an indication of how much NO_3^- was taken up by the plant root, while the total N concentration takes into account not only NO_3^-, but all the different types of N present in all the plant tissues. An interesting pattern that emerged from the total N data that was not seen in the short term NO_3^- uptake data was that mycorrhizas changed the shoot:root N content of plants with different CO_2 concentrations. Furthermore, the export of NO_3^- was associated with proline production in salinity stressed plants. The increase in proline concentration of mycorrhizal and non-mycorrhizal plants (Figure 2.2) was associated with a higher ratio of NO_3^- transported in the xylem stream of salinity stressed plants (Figure 3.3). This increase, together with an increased leaf NR activity (Figure 3.5) could have contributed to the increased proline production. The decreased proline production in the absence of salinity stress could be associated with a higher ratio of reduced N in the xylem sap of plants.

In this investigation, the role of elevated concentrations of CO_2 was different from the role reported by other authors working with elevated CO_2 and the effect on the physiology of non-mycorrhizal salinity stress plants. Cramer *et al.* (1995) concluded from their experiments with hydroponically grown tomato plants that elevated concentrations of DIC in the root zone increased NO_3^- uptake under conditions of salinity stress. In this investigation, elevated DIC in the root zone did not increase NO_3^- uptake, possibly because plants were grown under different experimental conditions. Cramer and Lips (1995) grew plants with a relatively higher P concentration (2 mM) in contrast to a very low P concentration (50 μM) that was used in this study. In the current investigation, the decrease in the plant dry weight of non-mycorrhizal plants supplied with elevated concentration CO_2 and 0 mM NaCl compared to plants at ambient CO_2 was also reported by Cramer and Lips (1995). However, at elevated concentrations of CO_2, non-mycorrhizal plants had a decreased plant dry weight at 75 mM NaCl compared to plants at 360 ppm CO_2, in contrast with results of Cramer and Lips (1995) where an increase in plant dry weight was reported. These differences in results could be the consequence of elevated concentrations of DIC stimulating plant growth under conditions of salinity stress (Cramer and Lips, 1995).

In this investigation, the O_2 consumption of mycorrhizal compared to non-mycorrhizal roots did not change, although arbuscular mycorrhizal infection have been reported to increase the respiration rate of plant roots (Baas *et al.*, 1989). Hawkins *et al.* (1999) also reported that arbuscular mycorrhizal infection did not change the root O_2 consumption of hydroponically grown wheat plants. The increase in the NO_3^- uptake rate of plants supplied with elevated concentrations of CO_2 in the absence of salinity, could not be associated with the rate of O_2 consumption, but the rate of CO_2 release was decreased at elevated concentrations of CO_2. Carbon skeletons, derived from the incorporation of HCO_3^- through the activity of PEPc

(Cramer *et al*., 1996) could have been used, with the NO_3^- taken up, for the production of amino acids. In the presence of salinity, there was no difference in the NO_3^- uptake rate or the O_2 consumption of roots, although there was a decrease in the CO_2 release rate at elevated CO_2 concentrations. The anapleurotic carbon provided at elevated concentrations of CO_2 was possibly used for the production of compatible solutes such as proline.

Summary

The hypothesis stated was that elevated concentrations of DIC could improve the NO_3^- uptake rate of arbuscular mycorrhizal plants under conditions of salinity stress. Elevated concentrations of DIC did not increased the NO_3^- uptake rate of arbuscular mycorrhizal plants in the presence of salinity stress, but in the absence thereof. Arbuscular mycorrhizal infection did not improve the NO_3^- uptake rate of plants because plant roots grown in hydroponic culture were provided with ready access to nutrients. Arbuscular mycorrhizal infection may not have been of significant benefit to the plant in assisting with nutrient uptake, but in the presence of elevated CO_2, mycorrhizal fungi increased the plant dry weight. Elevated concentrations of DIC in the root zone stimulated arbuscular mycorrhizal growth under conditions of salinity stress and this could have contributed to the increased growth of arbuscular mycorrhizal plants under conditions of salinity stress.

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