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Arbuscular Mycorrhizal Fungi Enhance Tolerance of *Rosa multiflora* cv. Burr to Bicarbonate in Irrigation Water

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ABSTRACT

High bicarbonate (HCO$_3^-$) of irrigation water can be detrimental to plant growth in sustainable horticultural production systems. The ability of arbuscular mycorrhizal fungi (AMF), ZAC-19, (composed of *Glomus albidum*, *Glomus claroideum*, and *Glomus diaphanum*) to enhance tolerance to HCO$_3^-$ was tested on *Rosa multiflora* cv. Burr. Arbuscular mycorrhizal colonized and non-inoculated (non-AMF) plants were treated with 0, 2.5, 5, and 10 mM HCO$_3^-$. Increasing HCO$_3^-$ concentration and associated high pH and electrical conductivity (EC) — reduced plant growth, nutrient uptake, and acid phosphatase activity, while increasing alkaline phosphatase activity (ALP). Inoculation with AMF enhanced plant tolerance to HCO$_3^-$, as indicated by greater growth (leaf, stem, and total plant dry weight, leaf area and leaf area ratio), leaf elemental concentration [nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), iron (Fe), zinc (Zn), aluminum (Al), boron (B)], leaf chlorophyll concentration, higher mycorrhizal inoculation effect, lower root Fe reductase activity, and generally lower soluble ALP activity. While AMF colonization was reduced by increasing HCO$_3^-$ concentration, colonization still occurred at high HCO$_3^-$ concentration. At 2.5 mM HCO$_3^-$, AMF plant growth was comparable to plants at 0 mM HCO$_3^-$, further indicating the beneficial effect of AMF for alleviation of HCO$_3^-$ plant stress.

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INTRODUCTION

Sustainable horticultural production systems will increasingly have to preserve and augment water resources through enhanced water use efficiency and utilization of non-conventional water resources. This may include utilization of marginal quality water, high in total dissolved solids (non-volatile solutes) (Oster, 1994; Shalhevert, 1994). The potential high bicarbonate ($\text{HCO}_3^-$) concentration and associated high pH of this irrigation water may be detrimental to plant growth, due to its adverse effects on availability and solubility of nutrients (Marschner, 1995). Prolonged nutrient deficiency results in significant reductions in growth, yield, and marketability of plant material. Thus, sustainable horticultural production will increasingly have to incorporate economically feasible and environmentally sound solutions to problems associated with high levels of $\text{HCO}_3^-$ in irrigation water.

Arbuscular mycorrhizal fungi [(AMF); Division Zygomycota/ Glomeromycota, Order Glomales/Glomerales (Morton and Benny, 1990; Schüßler et al., 2001)] exist in symbiotic associations with the fine young roots of higher plants (Smith and Read, 1997). Studies have demonstrated that AMF enhance plant nutrient acquisition [phosphorus (P), nitrogen (N), zinc (Zn), copper (Cu), and other ions] (Clark and Zeto, 2000), water relations (Augé, 2001), and alleviate cultural and environmental stresses (Jeffries et al., 2003) through greater effective root area and penetration of substrate(s), and activation and excretion of various enzymes by AMF roots and/or hyphae (Marschner, 1995; Smith and Read, 1997).

Therefore, we proposed a model study to determine if inoculation of plant material with AMF would enhance plant growth and nutrient acquisition under high levels of $\text{HCO}_3^-$ in irrigation water. An important arbuscular mycorrhizal genus is *Glomus* spp. Tulasne & Tulasne, which colonize a variety of host species (Marschner, 1995; Smith and Read, 1997), including *Rosa multiflora* Thunb. ex J. Murr. (Davies, 1987; Davies et al., 1987). Rootstocks of *Rosa multiflora* are susceptible to growth stress on alkaline soils (Reed et al., 1992). Our hypothesis was that inoculation of *R. multiflora* cv. Burr stem cuttings with an AMF mixed *Glomus* species isolate (ZAC-19) would enhance plant growth and nutrient acquisition under high levels of $\text{HCO}_3^-$ in irrigation water. The objective of this research was to determine if ZAC-19 would enhance *R. multiflora* cv. Burr tolerance to $\text{HCO}_3^-$ stress as determined by plant growth and nutrient acquisition.
MATERIALS AND METHODS

Cultural Conditions and Plant Material

This study was conducted under glasshouse conditions at Texas A&M University, College Station, Texas. Average day/night temperature and relative humidity were 28.1 ± 0.2°C / 23.4 ± 0.1°C, and 66.1 ± 0.8%/ 83.1 ± 0.5%, respectively. Average PPFD measured daily at solar noon was 403.6 ± 28.0 µmol·m⁻²·s⁻¹.

*Rosa multiflora* cv. Burr stem cuttings were obtained from stock plants at the Texas A&M University Agricultural Research and Extension Center, Overton, Texas. Cuttings with axillary buds intact were trimmed to approximately 15 cm in length and treated with a 0.3% indole-3-butyric acid powder (Hormodin® 2) to promote rooting and planted in 90-mL black plastic cells with a peat-based substrate previously autoclaved on two consecutive days for 20 min·d⁻¹ at 121°C (144.8 kPa). Cuttings were manually fogged as required using reverse osmosis (RO) water with a pH 7.2 and EC 0.06 dS·m⁻¹, and fertigated 25 days after planting with a formulation of Long Ashton nutrient solution (LANS) (Hewitt, 1966), modified to supply 31 mg·L⁻¹ P as sodium dihydrogen phosphate (NaH₂PO₄·H₂O). Uniform rooted cuttings were selected 30 days after planting, their roots rinsed free of propagation substrate with reverse osmosis (RO) water, and transplanted into 1.4-L green plastic containers. The container substrate had a textural analysis of 92% sand, 4% silt, and 4% clay, low organic matter (0.08%), pH 6.9, electrical conductivity (EC) 0.12 dS·m⁻¹, and nutrient levels with the following µg·g⁻¹: 2 N, 1 P, 13 potassium (K), 267 calcium (Ca), 27 magnesium (Mg), 0.13 zinc (Zn), 2.79 iron (Fe), 0.46 manganese (Mn), 0.05 Cu, 368 sodium (Na), 23 sulfur (S), and 0.14 boron (B). The container substrate was previously steam pasteurized with aerated steam on two consecutive days for 3 h·d⁻¹ at 80°C.

Arbuscular Mycorrhizal Inoculation

Half the rooted cuttings were not inoculated (non-AMF). The remaining rooted cuttings were individually inoculated at transplanting with 10 g of soil inoculum containing approximately 700 spores of a mixed *Glomus* species isolate, ZAC-19: *Glomus albidum* Walker & Rhodes, *Glomus claroideum* Schenck & Smith, and *Glomus diaphanum* Morton & Walker (Chamizo et al., 1998). The ZAC-19 inoculum was applied directly to the dibble hole at transplanting and included hyphae and colonized root segments of *Carica papaya* L. used for isolate multiplication. The textural analysis of the inoculum was 85% sand, 5% silt, and 10% clay, low organic matter (0.2%), pH 8.1, EC 0.45 dS·m⁻¹, and nutrient levels of the following µg·g⁻¹: 4 N, 24 P, 125 K, 512 Ca, 38 Mg, 14.65 Zn, 2.44 Fe, 3.7 Mn, 0.11 Cu, 393 Na, 26 S, and 0.22 B.
The ZAC-19 isolate was originally collected from non-irrigated (annual precipitation of approximately 450 mm), low nutrient (16 µg·g⁻¹ P), and a low organic matter (1.1%) sandy-loam soil (pH 5.4) used for commercial Phaseolus vulgaris L. production in Zacatecas, México (Chamizo et al., 1998). The ZAC-19 was propagated under glasshouse conditions in C. papaya pot culture (Brundrett et al., 1996) in 1999 at Texas A&M University, College Station, Texas, and root balls were harvested and stored in the dark in a cold room at 2°C until used.

Transplanted R. multiflora rooted cuttings were irrigated with approximately 150 mL of LANS, modified to supply 31 mg·L⁻¹ P as NaH₂PO₄·H₂O and 5 mg·L⁻¹ Fe as ferric diethylenetriaminepentaacetic acid [Fe-(DTPA)], every 3 days for 12 days. Plants were irrigated to achieve approximately 20% leachate fraction by volume.

Bicarbonate Application

Twelve days after transplanting, plants were irrigated with approximately 300 mL of a formulation of LANS, modified to supply P (NaH₂PO₄·H₂O) at 31 mg·L⁻¹ P, Fe (Fe-DTPA) at 5 mg·L⁻¹ Fe, and HCO₃⁻ at 0, 2.5 (250 mg·L⁻¹ KHCO₃), 5 (500 mg·L⁻¹ KHCO₃), or 10 [600 mg·L⁻¹ KHCO₃, 220 mg·L⁻¹ NaHCO₃, and 120 mg·L⁻¹ ammonium bicarbonate (NH₄HCO₃)] mM HCO₃⁻ every 4 days for 28 days. Average solution pH of the four HCO₃⁻ concentrations were, respectively, 6.0 ± 0.1, 7.1 ± 0.1, 7.3 ± 0.1, and 7.6 ± 0.1, and EC (dS·m⁻¹) were, respectively, 2.0 ± 0.1, 2.1 ± 0.1, 1.9 ± 0.1, and 2.4 ± 0.2. Plants were fertigated to achieve approximately 20% leachate fraction by volume. Leachate was collected at each irrigation (n = 5) using the pour through method apparatus (Wright, 1987), and pH and EC were analyzed.

Assessment of Plant Growth, Leaf Nutrient Analysis, and Chlorophyll Concentration

Growth measurements were recorded at harvest (n = 9) 32 days after transplanting, and included leaf area, leaf, stem, root, and total plant dry weight (DW). Tissue samples were dried for 7 days at 70°C and DW recorded. Leaf area ratio (LAR) [leaf area (cm²)/[plant DW (g⁻¹)], and specific leaf area (SLA) [leaf area (cm²)/[leaf DW (g⁻¹)] were calculated. The mycorrhizal inoculation effect (MIE) was calculated by the equation: MIE (%) = (total DW of AMF plant-total DW of non-AMF plant)/(total DW of non-AMF plant)⁻¹ × 100 (Plenchette et al., 1983; Sylvia, 1994).

Physiologically mature leaves from nine randomly selected plants per treatment were collected at harvest and pooled (plants # 1 to # 3, plants # 4 to # 6, and plants # 7 to # 9) into three replicate samples (n = 3) and ground to pass
a 40-mesh screen for complete tissue analysis at the MDS Harris Laboratory Services, Lincoln, Nebr.

Leaf chlorophyll concentration was determined at harvest (n = 3), by extraction of chlorophyll with acetone (Harborne, 1998). Procedure was modified as follows, representative semi-mature leaflets were collected and surface area was determined. Leaflets were placed in 5 mL of 80% acetone and stored in the dark for 7 days at 4°C. Supernatant was quantified with a spectrophotometer at 645 and 663 nm, and compared to an 80% acetone blank standard. Total chlorophyll concentration was expressed as mg·cm² of leaflet area.

**Root Iron Reductase and Phosphatase Activity**

Root iron reductase activity was determined at harvest (n = 3), based on the formation of Fe(II)-BPDS (Bathophenanthroline-Disulfonic Acid) complex (Brüggemann and Moog, 1989; Rosenfield et al., 1991). Procedure was modified as follows, roots were rinsed in nanopure water and tips excised (approximately 0.5 cm), 1.2 g of root tissue per sample were immersed in 40 mL of Fe³⁺ reductase assay solution containing 5 mM Mes [2-(N-Morpholino)ethanesulfonic acid] (pH 5.5), 0.5 mM calcium sulfate (CaSO₄·2H₂O), 0.1 mM Fe³⁺EDTA, and 0.3 mM 4,7-Diphenyl-1,10-phenanthroline-disulfonic acid (BPDS). Samples were aerated and incubated in the dark at 21°C for 4 h. The appearance of Fe³⁺ BPDS was quantified with a spectrophotometer at 535 nm, and compared to a standard concentration series (0, 3.58, 7.15, 21.45, 35.75, and 57.2 μM) of authentic Fe(II)-BPDS.

Root acid phosphatase (ACP) and alkaline phosphatase (ALP) activity (soluble and extractable) were determined at harvest (n = 3), based on the hydrolysis of p-nitrophenyl phosphate (p-NPP) substrate to yield p-nitrophenol (p-NP) and inorganic phosphatase (Eivazi and Tabatabai, 1977; Tabatabai and Bremner, 1969). Procedure was modified as follows, roots were rinsed in nanopure water and tips were excised (approximately 0.5 cm), 100 mg of root tissue per sample were transferred into a 600 μL extraction modified universal buffer (MUB) [100 mM THAM (Tris(hydroxymethyl) aminomethane)], 100 mM maleic acid (Toxilic Acid; cis-Butenedioic Acid), 5 mM citric acid (C₆H₈O₇), and 100 mM boric acid (H₃BO₃) pH 5.5 for ACP and pH 9.0 for ALP. Extractable ACP and ALP root samples were macerated. All root samples were centrifuged at 13,000 g for 15 min at 4°C. The reaction mixture consisted of 400 μL of supernatant, and 150 μL of 0.003 M p-nitrophenyl phosphate disodium salt hexahydrate (NPP) (C₉H₆NO₆PNa₂·6H₂O). The mixture was then incubated at 37°C for 45 min for ACP and ALP. The reaction was stopped by the addition of 100 μL 500 mM calcium chloride (CaCl₂·2H₂O) and 400 μL 500 mM sodium hydroxide (NaOH). Precipitate was recovered by centrifugation at 13,000 g for 15 min. The p-NP concentration of the supernatant was quantified with a
spectrophotometer at 420 nm, and compared to a standard concentration series (0, 1.4, 2.8, 4.2, 5.6, and 7.0 mM) of authentic \( p \)-NP (C\(_6\)H\(_5\)NO\(_3\)).

Assessment of Arbuscular Mycorrhizal Development

For AMF analysis of roots, 1-cm root segments from three randomly selected plants per treatment were sampled at harvest and pooled to assess colonization percentage through clearing with potassium hydroxide (KOH), and staining of root samples with trypan blue (C\(_{34}\)H\(_{24}\)N\(_6\)O\(_{14}\)S\(_4\)Na\(_4\)) (Phillips and Hayman, 1970). Twenty-five 1-cm stained root pieces were placed on each slide and three observations (the top, the middle, and the bottom) per 1-cm root segments were made with a microscope at 40\( \times \). The presence of arbuscules, vesicles, and hyphae was determined (Biermann and Linderman, 1981). There were nine slides per treatment (\( n = 3 \), 675 observations per treatment from 225 1-cm root segments).

Statistical Design

The experiment was a 2 \( \times \) 4 factorial in a completely randomized design with two AMF levels (AMF and non-AMF) and four levels of HCO\(_3\) (0, 2.5, 5, and 10 mM HCO\(_3\)). There was one rooted \( R. \) multiflora cv. Burr cutting per container, with each container as a single replicate. Data were analyzed using Analysis of Variance (ANOVA) (SAS Institute Inc., 2000).

RESULTS

Leachate Analysis

Bicarbonate caused a significant increase in leachate pH and EC, which increased over the duration of the study regardless of AMF treatment. In non-AMF plants substrate pH was between 7.7 and 8.3 at the end of the experiment, while in AMF plants it was between 7.4 and 8.3. Electrical conductivity increased with increasing HCO\(_3\), from 3.8 to 5.0 dS-m\(^{-1}\) in non-AMF plants, and between 4.7 and 5.4 dS-m\(^{-1}\) in AMF plants.

Plant Growth Parameters

Bicarbonate caused a significant reduction in all plant growth parameters (Tables 1 and 2), but they were significantly increased by AMF, except for root DW and leaf number. The HCO\(_3\) \( \times \) AMF interaction was not significant for any growth parameters, except SLA.
Table 1

Effect of bicarbonate (HCO$_3^-$) and arbuscular mycorrhizal fungi (AMF) on growth of *Rosa multiflora* cv. Burr plants

<table>
<thead>
<tr>
<th>HCO$_3^-$ (mM)</th>
<th>AMF inoculation</th>
<th>Leaf DW (g)</th>
<th>Stem DW (g)</th>
<th>Root DW (g)</th>
<th>Total plant DW (g)</th>
<th>MIE$^z$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>4.4 ± 0.5$^y$</td>
<td>4.6 ± 0.4</td>
<td>0.9 ± 0.1</td>
<td>9.9 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>5.0 ± 0.3</td>
<td>4.6 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>10.6 ± 0.5</td>
<td>7</td>
</tr>
<tr>
<td>2.5</td>
<td>No</td>
<td>2.8 ± 0.3</td>
<td>3.5 ± 0.3</td>
<td>0.6 ± 0.1</td>
<td>6.9 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3.9 ± 0.3</td>
<td>4.3 ± 0.4</td>
<td>0.8 ± 0.1</td>
<td>8.9 ± 0.7</td>
<td>29</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>2.4 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>6.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3.1 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>7.4 ± 0.4</td>
<td>21</td>
</tr>
<tr>
<td>10</td>
<td>No</td>
<td>2.1 ± 0.1</td>
<td>2.9 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>5.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.6 ± 0.2</td>
<td>3.4 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>6.7 ± 0.5</td>
<td>18</td>
</tr>
</tbody>
</table>

Significance$^x$

<table>
<thead>
<tr>
<th></th>
<th>AMF</th>
<th>HCO$_3^-$</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>**</td>
<td>***</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^x$Significance according to ANOVA, NS, *, **, ***, nonsignificant and significant $P \leq 0.05, 0.01, 0.001$, respectively.

$^y$Means ± SE, (n = 9).

$^z$Mycorrhizal inoculation effect [MIE (%) = (total DW of AMF plant-total DW of non-AMF plant)/(total DW of non-AMF plant)$^{-1} \times 100$].

Increasing concentrations of HCO$_3^-$ significantly reduced total plant DW, stem DW, root DW, leaf DW (Table 1), leaf number, leaf area, SLA, and LAR (Table 2). Averaged over all HCO$_3^-$ concentrations, plants inoculated with AMF exhibited significantly greater values on all these parameters, except for root DW and leaf number, compared to non-AMF plants. The HCO$_3^-$ × AMF interaction was not significant, indicating that increasing HCO$_3^-$ stressed plants, regardless of AMF; however, AMF alleviated HCO$_3^-$ stress, as indicated by greater values in most plant growth parameters at all HCO$_3^-$ levels. This implies that plants inoculated with AMF had a greater tolerance to HCO$_3^-$ stress, and that arbuscular mycorrhiza partially alleviated HCO$_3^-$ stress at 2.5, 5, and 10 mM with increased plant growth, compared to non-AMF plants. The MIE was very low in plants subjected to 0 mM HCO$_3^-$ (Table 1), but it was greatly increased at 2.5 mM HCO$_3^-$. Higher concentrations of HCO$_3^-$ caused decreased MIE values.

Leaf Nutrient Analysis

In general, leaf nutrient concentration of N, P, Ca, Fe, Cu, and B was significantly reduced by increasing HCO$_3^-$ concentration, whereas K, Na, Mn, Mg, S, Zn, and Al were unaffected or had no consistent response (Tables 3 and 4). Conversely, levels of Mo increased with higher HCO$_3^-$ concentrations.
Table 2

Effect of bicarbonate (HCO$_3^-$) and arbuscular mycorrhizal fungi (AMF) on leaf growth of *Rosa multiflora* cv. Burr plants

<table>
<thead>
<tr>
<th>HCO$_3^-$ (mM)</th>
<th>AMF inoculation</th>
<th>Leaf area (cm$^2$)</th>
<th>SLA$^z$ (cm$^2$·g$^{-1}$)</th>
<th>LAR$^y$ (cm$^2$·g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No</td>
<td>58.2 ± 5.0$^x$</td>
<td>1088.0 ± 118.4</td>
<td>247.4 ± 14.3</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>54.1 ± 6.5</td>
<td>1162.3 ± 35.5</td>
<td>236.6 ± 21.3</td>
</tr>
<tr>
<td>2.5</td>
<td>No</td>
<td>49.4 ± 3.8</td>
<td>560.3 ± 56.1</td>
<td>203.7 ± 11.8</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>44.7 ± 3.9</td>
<td>878.2 ± 45.5</td>
<td>227.6 ± 21.9</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>44.6 ± 3.8</td>
<td>442.8 ± 58.9</td>
<td>183.6 ± 20.3</td>
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<tr>
<td></td>
<td>Yes</td>
<td>41.7 ± 3.6</td>
<td>669.1 ± 22.9</td>
<td>218.1 ± 11.9</td>
</tr>
<tr>
<td>10</td>
<td>No</td>
<td>38.2 ± 4.4</td>
<td>549.6 ± 54.5</td>
<td>206.5 ± 16.8</td>
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<tr>
<td></td>
<td>Yes</td>
<td>37.8 ± 4.5</td>
<td>412.3 ± 61.3</td>
<td>195.9 ± 20.3</td>
</tr>
</tbody>
</table>

Significance$^w$:

<table>
<thead>
<tr>
<th>HCO$_3^-$</th>
<th>AMF</th>
<th>SLA</th>
<th>LAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>2.5</td>
<td>**</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

---

$^z$Specific leaf area [leaf area (cm$^2$)]/leaf DW (g$^{-1}$).

$^y$Leaf area ratio [leaf area (cm$^2$)]/plant DW (g$^{-1}$).

$^x$Means ± SE (n = 9).

$^w$Significance according to ANOVA, NS, *, **, *** , nonsignificant and significant $P \leq 0.05$, 0.01, 0.001, respectively.

At selected concentrations of HCO$_3^-$, plants inoculated with AMF had significantly increased leaf nutrient concentration of N, P, K, Ca, Mg, S, Na, Fe, Zn, Cu, Al, B, and molybdeunum (Mo), compared to non-AMF plants. Neither AMF nor HCO$_3^-$ affected Mn levels (Table 4). The HCO$_3^-$ × AMF interaction was significant for Na, Fe, Zn, Cu, Al, and Mo.

In non-AMF plants, HCO$_3^-$ concentration induced decreased leaf nutrient concentration; however, there was no clear pattern as the HCO$_3^-$ concentration increased. The concentrations of nutrients more affected by HCO$_3^-$ were P, S, Fe, Zn, Cu, and Al. In AMF plants, HCO$_3^-$ induced decreased leaf nutrient concentration; however, there was no clear pattern as the HCO$_3^-$ concentration increased. The most affected nutrients were N, P, and S compared to AMF control plants.

Inoculated plants had a greater leaf N, P, and S concentration compared to non-AMF plants. At 2.5 mM HCO$_3^-$ concentration, AMF plants exhibited greatest leaf N, P and S concentration compared to non-AMF plants. Leaf K, Ca, Mg, Fe, and B concentration remained constant in inoculated plants treated with 2.5 mM HCO$_3^-$ compared to control AMF plants. At 2.5 mM HCO$_3^-$ concentration AMF plants exhibited greatest leaf K, Ca, Mg, Fe, and B concentration compared to non-AMF plants. Bicarbonate concentrations >2.5 mM decreased leaf K, Ca, Mg, Fe, and B concentration.
Table 3
Effect of bicarbonate (HCO$_3^-$) and arbuscular mycorrhizal fungi (AMF) on leaf macronutrient and Na concentration of *Rosa multiflora* cv. Burr plants

<table>
<thead>
<tr>
<th>HCO$_3^-$ (mM)</th>
<th>AMF inoculation</th>
<th>N (g·kg$^{-1}$)</th>
<th>P (g·kg$^{-1}$)</th>
<th>K (g·kg$^{-1}$)</th>
<th>Ca (g·kg$^{-1}$)</th>
<th>Mg (g·kg$^{-1}$)</th>
<th>S (g·kg$^{-1}$)</th>
<th>Na (g·kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No</td>
<td>32.4 ± 3.1z</td>
<td>2.4 ± 0.3</td>
<td>24.0 ± 2.2</td>
<td>6.9 ± 0.7</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>36.3 ± 0.3</td>
<td>2.6 ± 0.1</td>
<td>27.1 ± 0.8</td>
<td>7.3 ± 0.2</td>
<td>2.2 ± 0.0</td>
<td>2.3 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>2.5</td>
<td>No</td>
<td>22.1 ± 0.9</td>
<td>1.5 ± 0.1</td>
<td>15.5 ± 1.6</td>
<td>4.6 ± 0.5</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1</td>
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<td>7.4 ± 0.9</td>
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<td>0.3 ± 0.0</td>
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<td>5</td>
<td>No</td>
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<td>16.7 ± 1.9</td>
<td>4.4 ± 0.4</td>
<td>1.4 ± 0.1</td>
<td>1.0 ± 0.1</td>
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<td>1.4 ± 0.1</td>
<td>23.8 ± 1.7</td>
<td>6.1 ± 0.5</td>
<td>1.8 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>0.4 ± 0.0</td>
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<tr>
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<td>1.0 ± 0.1</td>
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<td>4.8 ± 0.8</td>
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<td>1.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
</tbody>
</table>

Significance* 
| AMF | ** | ** | ** | ** | ** | ** | ** | NS |
| HCO$_3^-$ | *** | *** | NS | * | * | * | * | NS |
| Interaction | NS | NS | NS | NS | NS | NS | NS | * |

z Means ± SE, (n = 3).

y Significance according to ANOVA, NS, *, **, ***; nonsignificant and significant $P \leq 0.05, 0.01, 0.001$, respectively.
Table 4
Effect of bicarbonate (HCO$_3^-$) and arbuscular mycorrhizal fungi (AMF) on leaf micronutrient concentration of *Rosa multiflora* cv. Burr plants

<table>
<thead>
<tr>
<th>HCO$_3^-$ (mM)</th>
<th>AMF inoculation</th>
<th>Fe (µg·g$^{-1}$)</th>
<th>Mn (µg·g$^{-1}$)</th>
<th>Zn (µg·g$^{-1}$)</th>
<th>Cu (µg·g$^{-1}$)</th>
<th>Al (µg·g$^{-1}$)</th>
<th>B (µg·g$^{-1}$)</th>
<th>Mo (µg·g$^{-1}$)</th>
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<tbody>
<tr>
<td>0</td>
<td>No</td>
<td>66.9 ± 3.2$^z$</td>
<td>35.3 ± 3.7</td>
<td>10.7 ± 0.6</td>
<td>3.5 ± 0.5</td>
<td>16.9 ± 0.5</td>
<td>90.3 ± 9.4</td>
<td>2.6 ± 1.0</td>
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<tr>
<td></td>
<td>Yes</td>
<td>70.1 ± 2.7</td>
<td>34.7 ± 1.3</td>
<td>12.7 ± 0.4</td>
<td>3.9 ± 0.0</td>
<td>15.3 ± 0.7</td>
<td>99.6 ± 2.6</td>
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<td>56.5 ± 5.8</td>
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<td>69.0 ± 6.5</td>
<td>32.8 ± 4.2</td>
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<td>4.2 ± 0.7</td>
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<td>98.8 ± 9.7</td>
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<td>16.9 ± 2.4</td>
<td>64.2 ± 5.8</td>
<td>11.0 ± 1.3</td>
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Significance$^x$

<table>
<thead>
<tr>
<th></th>
<th>AMF</th>
<th>HCO$_3^-$</th>
<th>Interaction</th>
<th></th>
</tr>
</thead>
<tbody>
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<td>***</td>
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<td>*</td>
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<tr>
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<td>***</td>
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<tr>
<td></td>
<td>NS</td>
<td>*</td>
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<tr>
<td></td>
<td>NS</td>
<td>**</td>
<td></td>
<td>NS</td>
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</table>

$^z$Means ± SE, (n = 3).

$^x$Significance according to ANOVA, NS, *, **, ***; nonsignificant and significant $P \leq 0.05$, 0.01, 0.001, respectively.
At 2.5 mM HCO$_3^-$, AMF plants exhibited greater leaf Zn and Cu concentration compared to control AMF and non-AMF plants. Zinc concentration remained constant with increasing HCO$_3^-$ concentration in AMF plants, however, in AMF plants leaf Cu concentration decreased with increasing HCO$_3^-$ concentration.

**Physiological Parameters**

Total leaf chlorophyll concentration of AMF and non-AMF was significantly reduced by increasing HCO$_3^-$ concentration (Figure 1), however, AMF plants had greater chlorophyll concentration at all HCO$_3^-$ concentrations, as suggested by the significant HCO$_3^-$ × AMF interaction.

![Figure 1](image-url)  
*Figure 1.* Effect of bicarbonate (HCO$_3^-$) and arbuscular mycorrhizal fungi (AMF) on total leaf chlorophyll concentration of *Rosa multiflora* cv. Burr plants. Treatment effects of HCO$_3^-$, AMF, and the interaction of AMF × HCO$_3^-$ were significant, at $P \leq 0.001$, 0.001, and 0.01, respectively. Means ± SE, (n = 3).
Figure 2. Effect of bicarbonate (HCO$_3^-$) and arbuscular mycorrhizal fungi (AMF) on root Fe reductase activity in *Rosa multiflora* cv. Burr plants. Treatment effect of AMF was significant (*P* ≤ 0.01). Bicarbonate and the interaction of AMF × HCO$_3^-$ were nonsignificant. Means ± SE, (n = 3).

The Fe reductase activity was not significantly reduced by increasing HCO$_3^-$ concentration (Figure 2). On average, AMF plants had significantly lower Fe reductase activity compared to non-AMF plants. The HCO$_3^-$ × AMF interaction was not significant.

The soluble ACP activity was significantly decreased by increasing HCO$_3^-$ concentration (Figure 3A), but the AMF and HCO$_3^-$ × AMF interaction were not significant. In AMF plants treated with 0 mM HCO$_3^-$ there was greater enzymatic activity compared to non-AMF control plants.

The extractable ACP activity was also significantly decreased by increasing HCO$_3^-$ concentration (Figure 3B). Plants inoculated with AMF treated with 0 mM HCO$_3^-$ exhibited a significant decrease in enzymatic activity compared to non-AMF control plants. The HCO$_3^-$ × AMF interaction was significant. In non-AMF plants treated with 0 and 2.5 mM HCO$_3^-$ there was greater enzymatic activity compared to AMF plants.
Figure 3. Effect of bicarbonate (HCO$_3^-$) and arbuscular mycorrhizal fungi (AMF) on root acid phosphatase (ACP) activity in *Rosa multiflora* cv. Burr plants. (A) Soluble ACP activity. Treatment effect of HCO$_3^-$ was significant ($P \leq 0.001$), while AMF and the interaction of AMF $\times$ HCO$_3^-$ was nonsignificant. Means $\pm$ SE, ($n = 3$). (B) Extractable ACP activity. Treatment effects of HCO$_3^-$, AMF, and the interaction of AMF $\times$ HCO$_3^-$ were significant at $P \leq 0.01$, 0.01, and 0.05, respectively. Means $\pm$ SE, ($n = 3$).
Soluble ALP activity was not significantly affected by increasing HCO$_3^-$ concentration or AMF inoculation (Figure 4A). The HCO$_3^-$ × AMF interaction was significant. In non-AMF plants treated from 0 to 5 mM HCO$_3^-$ there was increased enzymatic activity. At higher HCO$_3^-$ concentration there was a decline in the enzymatic activity, however enzymatic activity of AMF plants increased with increasing HCO$_3^-$ concentrations.

Extractable ALP activity was significantly increased by increasing HCO$_3^-$ concentrations (Figure 4B), but the AMF and HCO$_3^-$ × AMF interaction were not significant. In general, non-AMF plants exhibited greater enzymatic activity than AMF plants. In AMF and non-AMF plants treated from 0 to 5 mM HCO$_3^-$ there was increased enzymatic activity compared to control. At higher HCO$_3^-$ concentration (>5 mM HCO$_3^-$) enzymatic activity plateaued.

**Arbuscular Mycorrhizal Fungi Colonization**

No AMF colonization occurred with non-inoculated plants. Increasing HCO$_3^-$ concentration decreased AMF colonization, however colonization was still achieved (Table 5). Total colonization and hyphae levels ranged from 40% to 7.6%, respectively at 0 to 10 mM HCO$_3^-$ . Inoculated plants treated with 2.5, 5, and 10 mM HCO$_3^-$ exhibited significantly decreased arbuscule formation, and hyphae and total colonization compared to control AMF plants. No vesicles were observed in any treatments.

**DISCUSSION**

Plants treated with increasing HCO$_3^-$ concentrations exhibited significantly inhibited growth, may be caused by the inhibitory effect of HCO$_3^-$ on metabolic processes and/or impairment of root activity/growth (Alhendawi et al., 1997; Kosegarten et al., 1999) and/or nutrient solubility (Alcántara et al., 1988; Pearce et al., 1999). Although plant species and cultivars may differ in their tolerance to HCO$_3^-$ stress, root physiology and nutrient solubility are affected by the buffering capacity of HCO$_3^-$, which is related to an increase in substrate pH.

Arbuscular mycorrhizal colonization (arbuscules and hyphae) was adversely affected by increasing HCO$_3^-$ concentrations, which may be due to the high pH associated with increasing HCO$_3^-$ concentrations (Medeiros et al., 1994; van Aarle et al., 2002a). However, AMF colonization still occurred at the highest HCO$_3^-$ concentrations.

In general, AMF helped to partially alleviate HCO$_3^-$ stress, as indicated by greater plant growth compared to non-AMF plants. The MIE was low in plants treated with 0 mM HCO$_3^-$, suggesting that under non-stress conditions *R. multiflora* cv. Burr plants were only moderately AMF dependant (Bagyaraj et al., 1988; Plenchette et al., 1983). However, at ≥2.5 mM HCO$_3^-$, MIE values
Figure 4. Effect of bicarbonate (HCO$_3^-$) and arbuscular mycorrhizal fungi (AMF) on root alkaline phosphatase (ALP) activity in *Rosa multiflora* cv. Burr. (A) Soluble ALP activity. The interaction of AMF $\times$ HCO$_3^-$ was significant ($P \leq 0.05$), while HCO$_3^-$ and AMF were nonsignificant. Means $\pm$ SE, ($n = 3$). (B) Extractable ALP activity. The effect of HCO$_3^-$ was significant ($P \leq 0.01$), while AMF and the interaction of AMF $\times$ HCO$_3^-$ were nonsignificant. Means $\pm$ SE, ($n = 3$).
Table 5

Effect of bicarbonate (HCO₃⁻) on percentage arbuscules, hyphae, and vesicles in root cortical cells of arbuscular mycorrhizal (AMF) *Rosa multiflora* cv. Burr plants

<table>
<thead>
<tr>
<th>Bicarbonate (mM)</th>
<th>AMF inoculation</th>
<th>Arbuscules (%)</th>
<th>Vesicles (%)</th>
<th>Hyphae (%)</th>
<th>Total colonization (%)</th>
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<tr>
<td>0</td>
<td>Yes</td>
<td>5.0 ± 0.6</td>
<td>0.0</td>
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<td>2.5</td>
<td>Yes</td>
<td>1.8 ± 0.3</td>
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<td>26.1 ± 2.0</td>
<td>26.1 ± 2.0</td>
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<td>5</td>
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<td>1.5 ± 0.1</td>
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<td>15.4 ± 1.5</td>
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<td>10</td>
<td>Yes</td>
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<td>0.0</td>
<td>7.6 ± 0.7</td>
<td>7.6 ± 0.7</td>
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</table>

| Significance     | HCO₃⁻       | NS | *** | *** | *** |

Means ± standard error (n = 3, 675 observations per treatment from 225 1-cm root segments).

Significance according to ANOVA, NS, *, **, ****, nonsignificant and significant P ≤ 0.05, 0.01, 0.001, respectively.

increased, indicating that plants became more AMF dependent, as shown by the percent increase in total plant DW of AMF plants compared to non-AMF plants (Bagyaraj et al., 1988; Plenchette et al., 1983). Treatment with ≥5 mM HCO₃⁻ decreased MIE, compared to 2.5 mM HCO₃⁻, indicating that while AMF partially alleviated HCO₃⁻ stress, increasing HCO₃⁻ concentration also had a negative effect on AMF.

There was a nonsignificant trend of lower leaf number in AMF plants compared to non-AMF plants, however, leaf area, SLA, and LAR were higher in AMF plants treated with HCO₃⁻. This indicates that AMF plant leaves were thinner (generally higher SLA), but had a larger photosynthetic area per plant (greater LAR) in relative terms, probably as a result of carbon cost necessary to maintain AMF associations (Wright et al., 1998).

In general, AMF plants had significantly increased leaf nutrient concentration compared to non-AMF plants.Arbuscular mycorrhizal fungi enhance nutrient acquisition through greater effective root area and penetration of substrate(s) (direct access to nutrients outside the zone of nutrient depletion that develop close to roots and to nutrients in inaccessible microsites), and activation and excretion of various enzymes by AMF roots and/or hyphae (Clark and Zeto, 2000; Marschner, 1998). Arbuscular mycorrhizal fungi may tolerate adverse external pH conditions by modifying the pH of the mycorrhizosphere during nutrient uptake (Pacovsky, 1986).

It has been reported in many plant species that high alkalinity induces Fe deficiency due to reduced Fe availability in soil or uptake (Alhendawi et al., 1997; Römheld, 2000). Bicarbonate can also cause an increase in internal precipitation of Fe, rendering inactive Fe in the roots due to the alkalization of tissues (Römheld, 2000). In the present study, AMF plant leaves were deficient in Fe (Cabrera, 2003) at HCO₃⁻ concentrations >5 mM,
but in non-AMF plants Fe deficiency was detected at concentrations as low as 2.5 mM HCO$_3^-$, indicating that AMF contributed to the maintenance of adequate Fe nutrition under moderate alkalinity conditions. This implies that *Rosa multiflora* rootstock sensitivity to alkaline soils (Reed et al., 1992) can be alleviated by inoculation with AMF. Phosphorus and Zn plant acquisition can also be impaired due to decreased solubility at high alkalinity (De la Guardia and Alcántara, 2002; Yang et al., 1993). In our study, rose plants inoculated with AMF exhibited a remarkable improvement in both P and Zn leaf concentration, especially at 2.5 mM HCO$_3^-$, demonstrating the beneficial effects of root inoculation with AMF under moderate alkalinity stress. Increased P nutrition as a result of AMF associations may also indirectly increase uptake of other ions, including N, Cu, Fe, and Zn (Marschner and Dell, 1994).

Total chlorophyll concentration was significantly reduced by increasing HCO$_3^-$ concentrations, probably due to decreased Fe nutrition, as reported in many species. However, AMF plants had higher chlorophyll concentration at all HCO$_3^-$ concentrations compared to non-AMF plants, which may reflect the higher photosynthetic rate necessary to support the carbon cost of AMF associations (Trimble and Knowles, 1995; Wright et al., 1998). The majority of carbon to support the metabolism of AMF originates directly from host plant photosynthesis (Douds et al., 2000).

Increased photosynthesis of AMF plants may be mediated by enhanced Fe uptake, as Fe is essential for various plant metabolic reactions, including chlorophyll synthesis and photosynthesis (Marschner, 1995). Increased photosynthesis of AMF plants may also be mediated by increased P nutrition. Cytoplasmic inorganic phosphate (Pi) levels in leaves regulate carbon export, and thus photosynthesis via the triose-P/Pi translocator in the chloroplast membrane (Marschner, 1995). Low levels of Pi lead to a build up of starch in the chloroplast, which can decrease photosynthesis (Marschner, 1995). Pulse/chase experiments with $^{14}$CO$_2$ show a greater percentage of labeled photosynthates are transported out of leaves of AMF plants during the chase period compared to non-AMF plants (Douds et al., 1988).

In the present study, leaf Fe concentration decreased with increasing HCO$_3^-$ concentration. In strategy I plants Fe deficiency enhances the activation of plasma membrane-bound inducible reductase, enhances net excretion of protons, and enhances release of reductants/chelators as a means of alleviating Fe stress (Marschner and Römheld, 1994; Moog and Brüggemann, 1994). However, root Fe reductase activity was not significantly enhanced by increasing HCO$_3^-$ concentrations, suggesting that plant material was not Fe efficient under study conditions.

In general, AMF plants had significantly lower root Fe reductase activity and higher leaf Fe concentration compared to non-AMF plants, suggesting that AMF enhanced plant Fe uptake under HCO$_3^-$ stress. Arbuscular mycorrhizal fungi may enhance Fe uptake through the nutrient acquiring mechanisms
previously described (Caris et al., 1998). Arbuscular mycorrhizal fungi may produce Fe chelating compounds, for example siderophores (Cress et al., 1986), as do other fungi, including ectomycorrhizal fungi, to enhance Fe uptake (Leyval and Reid, 1991). However, in other studies the effect of AMF on Fe uptake is variable and inconsistent, and acquisition of Fe has been both enhanced (Al-Karaki and Clark, 1998; Al-Karaki et al., 1998) and reduced (Clark et al., 1999; Kothari et al., 1990) by AMF, depending on experimental conditions. Bacterial levels were not tested in this study, however AMF may indirectly stimulate bacterial populations (Bianciotto and Bonfante, 2002; Vázquez et al., 2000), which may enhance Fe availability and uptake (Carrillo-Castañeda et al., 2003; Cowart, 2002).

Biochemical and biophysical processes involved in P metabolism, and phosphatase synthesis, activity, and efficiency are inconsistent and not well understood (Ezawa et al., 2002; Joner et al., 2000; Tarafdar et al., 2001; van Aarle et al., 2002b). In this study, leaf P concentration decreased with increasing HCO$_3^-$ concentration, and increasing HCO$_3^-$ concentration decreased soluble and extractable ACP activity in AMF and non-AMF plants, suggesting that high HCO$_3^-$ concentration and associated high pH impaired ACP synthesis, release, and/or stability (Tabatabai, 1994). Conversely, increasing HCO$_3^-$ concentration and associated high pH resulted in increased soluble and extractable ALP activity in AMF and non-AMF plants, suggesting that high HCO$_3^-$ concentration and associated high pH had limited effect on ALP synthesis, release, and/or stability (Tabatabai, 1994). Bicarbonate concentrations $>5$ mM resulted in a sharp decrease in soluble ALP activity in non-AMF plants, suggesting that high HCO$_3^-$ concentrations and associated high pH are inhibitory to plant ALP synthesis, release, and/or stability. This may be attributed to: 1) lower Fe uptake potentially reducing chlorophyll synthesis and photosynthetic rates resulting in lower carbon accumulation and transport to the roots affecting ALP synthesis; 2) an internal alkalinization of root cell symplast impairing cell metabolism and affecting ALP synthesis, release, and/or stability; and 3) phosphorus precipitation with Ca limiting induction of ALP.

Arbuscular mycorrhizal plants had significantly lower soluble ALP activity at $<10$ mM HCO$_3^-$ and in general higher leaf P compared to non-AMF plants, suggesting AMF plants had enhanced P uptake and transport under HCO$_3^-$ stress. Arbuscular mycorrhizal fungi may also enhance P uptake through the nutrient acquiring mechanisms previously described (Bolan, 1991; Miyasaka and Habte, 2001). However, HCO$_3^-$ concentrations $>5$ mM induced a sharp increase in soluble ALP activity in AMF plants, suggesting that high HCO$_3^-$ concentrations and associated high pH were inhibitory to AMF P availability/uptake. As previously reported bacterial levels were not tested in this study, however AMF may indirectly stimulate bacterial populations (Bianciotto and Bonfante, 2002; Vázquez et al., 2000), which may enhance phosphatase activity, P availability, and uptake (Gryndler et al., 2002; Rodríguez and Fraga, 1999; Villegas and Fortin, 2002).
CONCLUSION

This report demonstrates that AMF enhance plant tolerance to HCO$_3^-$ stress, as indicated by the enhanced growth, nutrient uptake, leaf chlorophyll concentration, MIE, low Fe reductase activity, and low soluble ALP activity. At 2.5 mM HCO$_3^-$, AMF plant growth was comparable to non-AMF and AMF plants at 0 mM HCO$_3^-$, indicating the potential beneficial application of AMF for alleviation of HCO$_3^-$ stress in *Rosa multiflora*. It is suggested that if this study had been conducted using a commercial substrate with a higher buffering capacity, rather than the low buffering capacity of the sand substrate used in this study, the beneficial application of AMF for enhanced plant tolerance to HCO$_3^-$ stress may be greater at higher HCO$_3^-$ concentrations.

ACKNOWLEDGMENTS

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REFERENCES


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