INFLUENCE OF SOIL OXYGEN DEPLETION ON IRON UPTAKE AND REDUCTION IN MANGO (MANGIFERA INDICA L.) ROOTS

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Abstract. In alkaline agricultural soils, such as those in south Florida, a large amount of chelated iron must often be applied to tropical fruit trees, including mango (Mangifera indica L.), to avoid iron deficiency. In calcareous soil, long-term lack of oxygen (anoxia) often results in iron deficiency due to increasing amounts of bicarbonate generated as a result of anaerobic metabolism in the root and microbial respiration. Short-term anoxia of alkaline soils may increase iron uptake as a result of a large amount of chelated iron must be applied to the soil to prevent trees from suffering from iron deficiency. Applications of large quantities of chelated iron to tropical fruit orchards are a significant expense to growers.

In south Florida, tropical fruit trees are grown primarily in Krome Very Gravely Loam soil classified as loamy-skeletal, hyperthermic, Lithic Rendoll with a pH -7.2 to 8.2 (Noble et al., 1996). In these limestone soils, iron availability to plants is limited by the high soil pH due to precipitation of water insoluble iron hydroxides. Also, the high concentration of HCO₃⁻ in alkaline, calcareous soil reduces iron uptake in plants, possibly as a result of increased pH and decreased reductivity (redox) potential in the plant tissue, which can result in immobilization of iron (Römheld, 1986; Shi et al., 1993; Schmidt and Schuck, 1996). Avoidance of iron deficiency of plants in alkaline soils is not well understood and varies considerably among species and cultivars (Römheld and Marschner, 1983). Specific chelating agents, such as diethylene-triaminepentaacetic acid (DTPA) and ethylenediaminetetra-acetic acid (EDTA), form soluble complexes with iron in the soil, making it more readily available for plant uptake (Sievers and Bailar, 1962). For tropical fruit trees in alkaline soils, a large amount of chelated iron must be applied to the soil to prevent trees from suffering from iron deficiency. Applications of large quantities of chelated iron to tropical fruit orchards are a significant expense to growers.

Flooding is an increasing concern in Florida and other tropical and subtropical regions of the world where fruit crops are grown (Nuñez-Elisea et al., 1999). In agricultural areas of south Florida, episodic flooding is an increasing concern because the ecological restoration plan that is currently underway for the Everglades National Park will raise the water table in the area (Nuñez-Elisea et al., 1998). Low soil O₂ (anoxic) conditions as a result of flooding affects the physiology and growth of fruit trees and these effects are often related to the length of time that the soil is anoxic (Schaffer et al., 1992).

Long-term anoxia in the root zone leads to decreased iron uptake. When soils are low in oxygen for long periods of time, CO₂ concentration increases as a result of partially aerobic respiration and/or fermentation by roots, soil bacteria, and fungi. The CO₂ in the soil combines with H₂O to produce HCO₃⁻ as illustrated by the following equation: CO₂ + H₂O→H₂CO₃→H⁺ + HCO₃⁻. Limestone soils, such as those in south Florida tropical fruit orchards, are composed primarily of calcium carbonate (CaCO₃). When these limestone soils are flooded the increased CO₂ concentration (as a result of anaerobic fermentation by roots and soil microbes) combines with CaCO₃ and H₂O to form additional HCO₃⁻. Therefore, more HCO₃⁻ is produced as a result of anaerobiosis in calcareous soils compared to non-calcareous. The increased HCO₃⁻ concentration in the soil results in an immobilization of iron and reduced iron uptake by plants leading to iron chlorosis. High HCO₃⁻ concentrations in the soil result in iron immobilization in the plant (Mengel and Kirkby, 1982).

Short-term anoxia due to flooding leads to increased solubility of iron in the soil and a reduction of iron from the Fe³⁺ form to the Fe²⁺ form, which is more readily available for plant uptake (Larson et al., 1991; Ponnamperuma, 1966; Ponnamperuma, 1972). For mango trees in an alkaline soil, short-term anoxia can increase iron uptake (Zude et al., 1998).

Most iron in the soil is in the Fe₂⁺ form, which is not readily available to plants; however, roots have developed several mechanisms to increase iron availability. These mechanisms include: 1) soil acidification, which increases the solubility of Fe₂⁺, 2) reduction of Fe³⁺ to more soluble Fe²⁺ form, and 3) release of chelators by the roots, which form stable, soluble complexes with iron (Marschner, 1995). An enzyme in roots, iron reductase, reduces Fe³⁺ to Fe²⁺ (Bienfait et al., 1982), with the compounds nicotinamide adenine dinucleotide (NAD), NADP, roots.
cletide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as the required electron donors for the reaction (Sijmons et al., 1984; Buckhout et al., 1989; Brüggemann et al., 1990). Reduced aerobic root respiration, as a result of short-term soil anoxia, may inhibit oxidation of NAD(P)H to NAD(P)+, thereby increasing the reduction rate of Fe3+ to Fe2+.

The purpose of this study was to determine the effects of short-term soil anoxia on the capacity of mango (Mangifera indica L.) roots to reduce iron from Fe3+ to Fe2+ and the subsequent effect on iron uptake by the plants.

Material and Methods

Seedlings of the polyembryonic mango cultivar 'Kitchener' were grown in containers (filled with quartz sand) in a glasshouse at the Humboldt University, Berlin, Germany. In the glasshouse, day/night temperature was maintained at 25/20°C and plants received supplemental light (high pressure mercury lamp, HQGTS-400 W/D, Osram GmbH, Munich, Germany), which provided a photosynthetic photon flux of 600 μmol m⁻²s⁻¹. Twelve plants were supplied daily with an iron-free, alkaline nutrient solution (modified from Römheld and Marschner, 1983) four weeks before treatments were imposed, to obtain iron-deficient plants (-Fe) plants. The iron-free, alkaline nutrient solution plus Fe³⁺ sugar acid (7 mmol liter⁻¹). The -Fe plants were supplied with 12 other plants with the same nutrient solution to prevent iron stress as a result of excision (Bloom and Caldwell, 1988, Lambers et al., 1991).

Anoxia in the root zone was achieved by infusing N₂ into the root containers at a flow rate of 1 liter min⁻¹. The concentration of O₂ in the root containers was measured with a paramagnetic oxygen analyzer (PMA 10, M&C Instruments, Oosterhout, Netherlands) placed 10 cm below the surface of the substrate to determine if the rhizosphere was anoxic. Aerobic conditions were maintained by forcing ambient air through the root containers at the same flow rate as mentioned above. Root respiration was measured on 4 to 6 excised, branched root segments (5 cm long) with root tips intact. Root respiration (the net rate of CO₂ respired from the root) was measured in root segments that were enclosed in an air-tight cuvette (50 ml) connected to a CO₂ infrared gas analyzer (model CI301PS, CID, Inc., Vancouver, Washington). Respiration measurements were made in the dark in ambient air (399 μmol CO₂ mol⁻¹) with a flow rate into the cuvette of 300 ml min⁻¹. Temperature in the chamber during respiration determinations was 23 ± 1°C. Respirations of the root segments was determined within 5 to 10 min after steady-state conditions were reached, considerably less time than it takes for respiration to be inhibited by a wound response or desiccation as a result of excision (Bloom and Caldwell, 1988; Lambers, et al, 1991).

Root iron concentrations were determined in two samples from each of 3 trees (replications). Root tissue was oven dried and iron was acid-extracted (in 25% HCl, 10% HNO₃) from the dried root tissue. Root iron concentration was determined with an atomic absorption spectrometer (model 905AA, GBC Scientific Equipment Pty. Ltd., Victoria, Australia) at a wavelength of 372 nm.

Discriminative analysis of oxidized NAD(P)⁺ and reduced NAD(P)H in root tissue was achieved by acid and alkaline plant tissue extraction, respectively, since NAD(P)+ is only stable at low pH whereas NAD(P)H is stable at high pH. Concentrations of the oxidized and reduced pyridine nucleotides were extracted with perchloric acid (HClO₄) and potassium hydroxide (KOH), respectively and analyzed from fresh root samples (Brinkman et al., 1973; Zhao et al., 1987; Zude-Sasse and Lüdders, 2000). Samples were homogenized in an Ultra-Turrax® disperser (IKA Labortechnik, Staufen, Germany) and centrifuged (model Labofuge GH, Heraeus Instruments, Osterode, Germany) at 500 x gravity and the supernatant solutions were adjusted to pH 7.4 (with tris-HCl buffer, 60 mmol) and pH 7.6 (with tris-EDTA buffer, 3.9 mmol), respectively.

Concentrations of extracted reduced and oxidized NAD and NADP were determined separately via the specific enzymatic reactions with alcohol dehydrogenase and gluconate-6-phosphate dehydrogenase, respectively which reacted colorimetrically with 2,6-dichlorophenolindophenol-di hydrodye (DCPIP, 1.3 mmol) and n-methylidibenzopyrazine methyl sulfate (NMS, 7.5 mmol). The colorimetric reaction was measured at 625 nm (Karayannis and Siskos, 1982) with a spectrophotometer (SP8-300, TJA Solutions, Cambridge, U.K.). Redox potential and the change in free energy (ΔG; a measure of the potential chemical energy available to drive the conversion of NAD[P]H to NAD[P]⁺) were calculated for roots of iron-deficient plants in the anaerobic and aerobic root environment. The AG indicates the probability of the formation of the oxidized compound(s) NAD(P)⁺ in redox reactions (Sijmons et al., 1984; Schmidt et al., 1990; Wigge et al., 1993).

Results and Discussion

Infusing N₂ into the root chambers resulted in a rapid decline of the O₂ partial pressure in the rhizosphere. Within 30 sec, O₂ could no longer be detected in the root zone. Anoxia in the root zone for 28 hr led to a decrease in the root respiration rate in mango seedlings (Fig. 1). This was due to the absence of O₂ as the terminal electron acceptor in the chain of biochemical reactions of respiration. When the rhizosphere was anaerobic, the concentration of extracted NADH, the reduced form of the nucleotide, increased relative to the con-
Table 1. Influence of short-term (28 h) root zone anoxia on nucleotide concentrations in iron-sufficient (+Fe) and iron-deficient (-Fe) mango trees.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NADP⁺ (µmol 100 g fresh wt⁻¹)</th>
<th>NADPH (µmol 100 g fresh wt⁻¹)</th>
<th>NAD⁺ (µmol 100 g fresh wt⁻¹)</th>
<th>NADH (µmol 100 g fresh wt⁻¹)</th>
<th>NADPH/NADP⁺+NADPH (ratio)</th>
<th>NADH/NAD⁺+NADH (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic, +Fe</td>
<td>24.0 a</td>
<td>16.3 a</td>
<td>14.0 a</td>
<td>6.5 b</td>
<td>0.40 a</td>
<td>0.32 b</td>
</tr>
<tr>
<td>Anaerobic, +Fe</td>
<td>22.5 a</td>
<td>16.2 a</td>
<td>15.0 a</td>
<td>16.0 a</td>
<td>0.42 a</td>
<td>0.52 a</td>
</tr>
<tr>
<td>Aerobic, -Fe</td>
<td>25.2 a</td>
<td>17.5 a</td>
<td>15.8 a</td>
<td>9.0 b</td>
<td>0.41 a</td>
<td>0.36 b</td>
</tr>
<tr>
<td>Anaerobic, -Fe</td>
<td>24.0 a</td>
<td>16.6 a</td>
<td>16.0 a</td>
<td>17.4 a</td>
<td>0.41 a</td>
<td>0.48 a</td>
</tr>
</tbody>
</table>

Different letters within a column indicate significant differences among means (P ≤ 0.05).

The mean root iron concentration for plants in the anaerobic treatment of 240 mg Fe Kg⁻¹dw was significantly greater (according to a standard T-test, P ≤ 0.01) than that of plants in the aerobic treatment which was 190 mg Fe Kg⁻¹dw. Thus, short-term root zone anoxia enhanced iron concentration in mango roots. The lower iron concentration in the roots of plants in the aerobic compared to the anaerobic treatment may have been partially due to a dilution effect in the aerobic treatment due to more growth (Zude et al., 1998).

The more negative ΔG (Fig. 2) and the increased NADH concentration in mango roots as a result of root anoxia promoted iron reduction and uptake by supporting the reduction reaction of Fe³⁺ to Fe²⁺. This was already suggested in the literature (Schmidt et al., 1990; Szabó-Nagy and Erdei, 1993; Rabotti and Zocchi, 1994; Schmidt and Schuck, 1996). In an anaerobic root environment the activity of iron reductase (necessary for the reduction of Fe³⁺ to Fe²⁺) may be promoted by increased concentration of NADH, the electron donor for the reaction. In agreement with our observations of mango, previous studies with cucumber and Plantago lanceolata have indicated that NADH was the major electron donor for reduction of Fe³⁺ to Fe²⁺ (Schmidt et al. 1990; Rabotti and Zocchi, 1994). However, other studies with bean and sunflower plants indicated that NADPH rather than NADH was the major electron donor for iron reduction (Sijmons et al., 1984; Szabó-Nagy and Erdei, 1993). The contrasting results between experiments may have been due to differing O₂ concentration in the root environment resulting in different electron donors (NADH vs. NADPH) for iron reduction. In anaerobic root conditions the non-phosphorylated compound, NADH, is more available and is therefore used as the electron donor instead of the phosphorylated compound, NADPH.

In conclusion, increased iron uptake by mango roots as a result of short-term root zone anoxia may be due, at least in part, to increased reduction of Fe³⁺ (the form of iron most abundant in the soil, but not available for plant uptake) to Fe²⁺ (the form that is readily absorbed by plants). When the rhizosphere was anaerobic, increased iron reduction may have resulted from the increased root concentration of NADH, an electron donor for the iron reduction reaction. In previous studies, anoxia as a result of flooding increased the extractable iron concentration in a calcareous soil (Larson et al., 1991). Thus, short-term flooding may ameliorate iron deficiency in mango trees grown in alkaline soils by increasing iron solubility in the soil and the availability of iron to the plant.

Figure 2. Influence of root zone anoxia on the redox potential and change in free energy of NAD⁺ in mango roots. Anaerobic conditions are denoted with open symbols and dotted lines; squares indicate the redox potential, and triangles indicate the change in free energy.

Literature Cited


