

Iron Deficiency Induced Changes in Iron Reductase Activity in Papaya Roots

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ABSTRACT. Four papaya (*Carica papaya* L.) cultivars were cultured aeroponically or in perlite to determine the magnitude, timing, and root locality of Fe reductase induced by Fe deficiency. Five soybean [*Glycine max* (L.) Merrill] lines with a known range of Fe-deficiency chlorosis scores were cultured in perlite for comparison. Speed of inducement of Fe reductase activity was determined in plants cultured without Fe for 0 to 17 days. Location of Fe reductase activity was determined by sectioning roots from the tip to 60 to 70 mm proximal to the root tip from plants cultured without Fe for 16 to 19 days. The Fe reductase system was induced in all papaya cultivars after 7 to 11 days without Fe, and activity increased through 17 days. Iron reductase activity in all papaya cultivars was comparable to the most tolerant soybean line. The zone of highest activity was the apical 10 mm of roots. These results indicate that papaya roots are highly efficient in induced Fe reductase activity. The highest activity in root tips underscores the importance of maintaining a healthy, continually growing root system with numerous growing points when culturing papaya in alkaline substrates.

Iron deficiency induces physiological changes in the roots of some plants that increase Fe uptake from alkaline soils. Uptake of Fe into the cytoplasm is restricted to Fe²⁺ for dicots (Marschner, 1995), yet soil reaction maintains Fe as Fe³⁺ in alkaline soils. The physiological changes address these soil reaction issues, and are represented by a complex set of responses. Some of these responses include an Fe reductase system capable of reducing Fe³⁺ to Fe²⁺, release of reductants into the rhizosphere, an increase of organic acids with chelating properties, and release of protons which reduces Fe³⁺ to Fe²⁺ by directly influencing soil reaction (Landsberg, 1981; Marschner et al., 1986; Olsen and Brown, 1980; Römheld, 1987). For dicots, the inducible changes and resulting increase in Fe acquisition have been characterized as strategy I iron efficiency (Römheld and Marschner, 1986).

Papaya (*Carica papaya*) is cultivated throughout the equatorial regions, and has naturalized in many tropical lowland habitats. The optimum soil pH for papaya cultivation reportedly ranges from 5.5 to 7.0 (Awada et al., 1975; Marler et al., 1994). Papaya root growth is limited in acid soils, and correction for Ca deficiency and Al toxicity improves root growth (Marler and dela Cruz, 2001). Growth and morphology of papaya seedlings grown in complete nutrient solution were relatively unaffected within the pH range of 4.0 to 8.8 (Marler, 1998). Furthermore, Manshardt and Zee (1994) reported that calcareous soils characterize the center of origin for the species. Commercial production of papaya in calcareous soils occurs in southern Florida (Malo and Campbell, 1986) and northern Guam. We have not observed Fe deficiency symptoms on papaya plants growing in Guam, except following root damage by poor aeration or canopy damage by tropical cyclones.

Kannan (1985) reported that acidification of the rooting medium occurred when papaya was grown in Fe-deficient media. No other reports have included the extent of strategy I mechanisms associated with Fe efficiency for this species. Since papaya plants thrive in calcareous soils, we hypothesized that the species may

be endowed with an Fe reductase system induced by Fe deficiency which is a characteristic attribute of strategy I Fe efficiency. Therefore, the objective of this study was to determine the magnitude, timing, and root locality of the Fe reductase system for papaya roots.

Materials and Methods

PLANT MATERIAL AND CULTURE. Four papaya cultivars were used in the study, two from the Solo group ('Sunrise' and 'Waimanalo') and two from the Formosa group ('Tainung 2' and 'Red Lady'). Papaya breeding programs have focused primarily on fruit characters and more recently on disease resistance. Choice of cultivar in production is determined largely by target market in Guam. The Asian and Pacific Islands markets require the larger fruit of the Formosa group, which have been selected for fresh weight generally >1.5 kg. The remainder of the markets prefer the smaller fruit of the Solo group, which are up to ≈0.5 kg in weight. To our knowledge, there have been no observations of variations among papaya cultivars in terms of soil chemistry or response to calcareous soils. The two cultivars representing each group were selected because they are highly characteristic of their group and are widely planted.

Seeds of the four cultivars were sown on 2 Feb. 1999 in flats with 7.5-cm-diameter cells (280 mL capacity) using Sunshine Mix 4 (Sun Gro Hort., Bellevue, Wash.). Germination and growth through 25 Mar. 1999 occurred under a polypropylene covered shelter with 90% to 92% sunlight transmission. The plants received a weekly fertilization drench of a complete nutrient solution based on 7.5 mm nitrogen (Peter's Excel; Teufel Nursery, Inc., Woodinville, Wash.). Mean daily air temperature extremes were 24 ± 2 °C and 33 ± 1 °C.

Plants were divided into two groups on 25 Mar. 1999 for separate experiments conducted underneath the same polypropylene rain shelter. One group was bare-rooted and transferred to aeroponics where roots were misted with half-strength Hoagland's solution (pH ≈6.5) (Hoagland and Arnon, 1950). The systems were comprised of 150-L containers containing ≈30 L of solution. Submersible pumps were used to supply suspended mist nozzles with the nutrient solution such that the entire volume of the aeroponics system was continuously being sprayed with the solution. Plants were held in place with foam after inserting the

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roots into holes on the top of the chambers. The plants were arranged in a randomized complete block with eight replications. Iron was supplied in the solution at $5 \text{ mg}\cdot\text{L}^{-1}$ as Fe-EDDHA [ethylenediamine di (O-hydroxyphenyl acetic acid)]. The solution was switched to half-strength Hoagland's solution without Fe on 1 Apr. 1999, after running several changes of reverse osmosis water through the aeroponics system for 12 h. Thereafter, the minus-Fe solution was changed on weekly intervals. The reductase assay was conducted on 16, 18, and 19 d of growth in the minus-Fe solution.

Each plant in the second group was replanted into a 625-mL plastic container filled with washed perlite. Each container was drenched with half-strength Hoagland's solution plus Fe on a daily basis. On 3 Apr. and at 3, 6, 10, and 13 d later, four plants per cultivar were switched to receiving daily drenching of half-strength Hoagland's solution minus Fe. Four plants per cultivar, designated as control plants, received the complete solution until the day of the assay. Each container was flushed with reverse osmosis water at least 10 times throughout the photoperiod on the day the switch from plus-Fe to minus-Fe solution occurred. The reductase assay was conducted on 20 Apr. 1999 after plants had grown without Fe for 0, 4, 7, 11, 14, or 17 d. These plants were arranged in a randomized complete block design ($n = 4$). We used this approach to determine the timing of Fe reductase activity because switching individual replications to minus-Fe was not possible in the aeroponics systems.

We also included several soybean (*Glycine max*) cultivars to provide a frame of reference for the papaya data, since the Fe reductase activity for soybean has been heavily studied and that for papaya has not been reported previously. Furthermore, Fe reductase activity for soybean is highly correlated with visual scores of Fe-deficiency chlorosis (Jolley et al., 1992). Five soybean breeding lines from Novartis Seeds, Inc. (Golden Valley, Minn.) were used, based on a range of Fe-deficiency chlorosis ratings from multiple years and sites. Based on their data, two soybean lines were tolerant (S66-90 and S83-30), two were intermediate (S57-11 and RA452), and one was sensitive (S75-55) (Howard Gabe, personal communication). Seeds were planted directly in washed perlite in 625-mL plastic containers on 1 Apr. 1999, and received the complete nutrient solution drench until 9 Apr. Thereafter, the plants received the minus-Fe solution until 20 Apr. when the assay was conducted. The plants were arranged in a randomized complete block design ($n = 4$).

IRON REDUCTASE ASSAY. The entire root systems of plants from the aeroponics system were harvested and rinsed in distilled water. The plants were harvested by block. Four types of root segments were assayed. Primary lateral roots grew $\geq 20 \text{ mm}\cdot\text{d}^{-1}$, and developed short, thin secondary roots (10 to 20 mm long). One root segment assayed was the terminal 10 mm of these short secondary roots. The other three segments assayed were based on distance from the tip of the primary lateral roots (0 to 10, 30 to 40, and 60 to 70 mm). The number of roots included from each plant was 6 to 7 for the 3 segments from the primary lateral roots, and 10 to 12 for the secondary roots. After four blocks were assayed, it was apparent that the Fe reductase activity in the 0 to 10 mm segment declined to a relatively low level by the 30 to 40 mm segment. Thus, we modified the methods for the four remaining blocks to characterize more fully the Fe reductase activity near the root tip. The segments prepared for assay from these plants were 0 to 5, 5 to 10, 10 to 15, and 15 to 20 mm. The number of roots included from each plant was 10 to 12.

Papaya and soybean plants cultured in perlite were bare-

rooted and the intact root system was rinsed in distilled water before excising 6 to 7 papaya or 10 to 12 soybean primary root tips of 10 mm length. These plants were harvested by block.

Root sections were rinsed in distilled water before assay using methods similar to Albano and Miller (1996). They were bathed in a 0.2 mM CaSO_4 solution for 10 min then immersed in 3 mL oxygenated assay solution containing 0.2 mM CaSO_4 , 0.1 mM $\text{Fe}^{3+}\text{EDTA}$ (ethylenediamine-tetraacetic acid), and 0.3 mM of the ferrous chelator BPDS (4,7-diphenyl-1,10-phenanthroline-disulfonic acid). The solution was buffered at pH 5.5 with 5 mM MES [2-(N-morpholino) ethanesulfonic acid]. Assays were conducted in the dark at 23 °C. The appearance of Fe^{2+} -BPDS in the assay solution was determined at 120 min by absorbance at 535 nm (DR-200; Hach, Loveland, Colo.). Blanks (assay solution minus roots) were assayed at least every hour to quantify autoreduction (background Fe reduction of the assay conditions). The fresh weight (FW) of the roots in each sample was determined at the end of the incubation period. The assays were conducted between 0900 and 1800 HR, and the assays for each block were completed in ≤ 2 h.

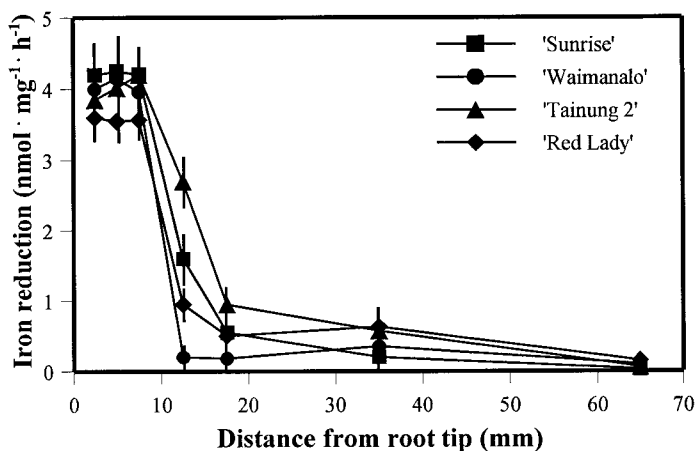


Fig. 1. Iron reduction of excised root segments of four papaya cultivars grown for 16 to 19 d without Fe in aeroponics culture as a function of distance from the root tip. Vertical bars indicate SE ($n = 4$).

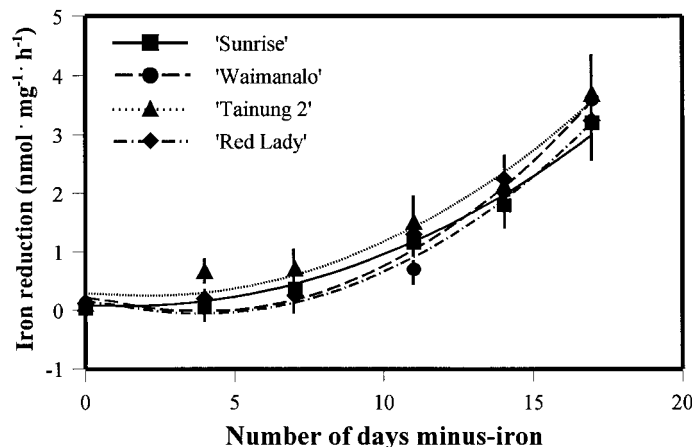


Fig. 2. Iron reduction of excised root tips (10 mm) from four papaya cultivars growing in perlite as a function of days without Fe supplied to the roots. Regression lines: for 'Red Lady', Fe reduction = $0.221 - 0.144 \times d + 0.0188 \times d^2$, $r^2 = 0.90$; for 'Sunrise', Fe reduction = $0.101 - 0.034 \times d + 0.0120 \times d^2$, $r^2 = 0.88$; for 'Tainung 2', Fe reduction = $0.317 - 0.064 \times d + 0.0150 \times d^2$, $r^2 = 0.93$; and for 'Waimanalo', Fe reduction = $0.291 - 0.161 \times d + 0.0208 \times d^2$, $r^2 = 0.98$. Vertical bars indicate SE ($n = 4$).

CALCULATIONS AND STATISTICS. The concentration of Fe²⁺-BPDS produced was calculated using an extinction coefficient of 22.14/mm per cm (Welch et al., 1993). Autoreduction was minimal, but was included in calculations by averaging two sequential blanks and subtracting this mean from each root assay which fell between the two blanks. Total reduction per 3 mL of solution was standardized to a unit root FW included in each assay. Total root FW ranged from 8 to 20 mg for short, secondary papaya roots, 15 to 60 mg for primary papaya roots, and 20 to 50 mg for soybean roots.

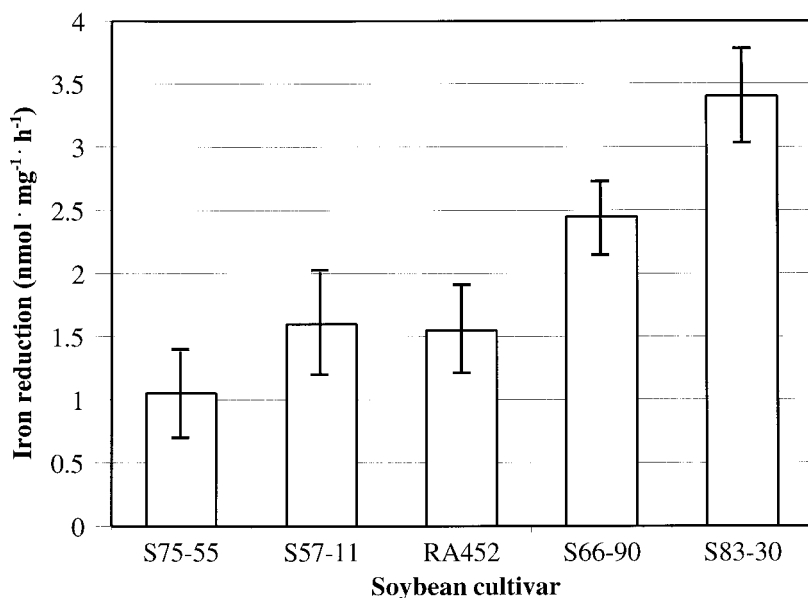
The pattern of Fe reduction as a function of culture time without Fe was determined using regression analysis, with the number of days defined as the independent variable. The resulting curves for the four cultivars were represented by quadratic equations. Equations were tested by analysis of covariance for homogeneity among the cultivars. Papaya root Fe reduction data are expressed as a function of root locality and soybean root Fe reduction data are presented as means of four replications ± SE.

Results

We did not observe any canopy symptoms indicative of Fe deficiency in any of the papaya plants. Iron reduction in the terminal 5 mm of papaya lateral roots in the aeroponics study was 3.5 to 4.2 nmol·mg⁻¹·h⁻¹, and no differences occurred among the four cultivars (Fig. 1). Iron reduction remained high in the 5 to 10 mm section, but declined by ≈5% for 'Waimanalo' to ≈65% for 'Tainung 2' at 10 to 15 mm from the root apex. Iron reduction potential declined more in the 15 to 20 mm section, ranging from 5% to 25% of the 0 to 5 mm values. Iron reduction was negligible at 60 to 70 mm from the root terminal.

Iron reduction potential in the short secondary root tips was slightly lower than in primary root tips (data not presented), and ranged from 2.3 ± 0.5 nmol·mg⁻¹·h⁻¹ for 'Red Lady' to 4.3 ± 0.6 nmol·mg⁻¹·h⁻¹ for 'Sunrise'. Values exceeded Fe reduction levels found in primary lateral roots beyond 10 mm from the tip.

Fig. 3. Iron reduction of excised root tips (10 mm) from five soybean cultivars with a range of Fe-deficiency chlorosis when grown in alkaline soils. Cultivars were grown in perlite for 11 d without Fe supplied to the roots. Vertical bars indicate SE (n = 4).



Development for induced reductase activity as a function of time fit quadratic equations (Fig. 2). Analysis of covariance test of homogeneity of the equations indicated the four equations could not be used to describe the same response curve ($P \leq 0.029$). Thus, the timing of initial inducement of the reductase system differed among the cultivars.

Iron reduction increased in 'Tainung 2' roots 4 d after culture without Fe, and increased in roots of all four cultivars by day 11 (Fig. 2). Iron reduction in 'Waimanalo' roots increased more slowly than in the other three cultivars, but by day 14, the four cultivars were similar.

Iron reduction for the soybean cultivars followed the same pattern as the visual Fe-chlorosis scores from previous field trials. The cultivar with the worst chlorosis scores (S75-55) exhibited a reduction potential of only 1 nmol·mg⁻¹·h⁻¹, the two with the best chlorosis scores (S66-90 and S83-30) exhibited the highest reduction potential, and the two with the intermediate chlorosis scores (S57-11 and RA 452) exhibited intermediate reduction potential (Fig. 3). Iron deficiency chlorosis was apparent in the S75-55, S57-11, and RA 452 plants by day 5 of induction.

Discussion

Papaya roots responded to Fe deficiency with enhanced Fe reduction. Induction was slower than in efficient soybean cultivars (Jolley et al., 1992), but the magnitude under the conditions of this study was comparable with that of the efficient soybean line S83-30 and greater than the other four soybean lines (Fig. 3). If these four cultivars are representative of the species, papaya roots are highly efficient at Fe reduction. There were no obvious differences between the Formosa cultivars and the Solo cultivars. Thus, both breeding programs appear to have retained root reductase potential throughout their selection processes.

The short lateral roots were highly active in Fe reduction. These short thin roots are ephemeral, typically lasting 1 to 2 weeks on plants growing in mineral soil (T. Marler, unpublished data). Our results indicate that these ephemeral roots play an important role in Fe nutrition during their short life span, as they allow any given length of pioneer root to mine a greater volume of soil during the temporary increase in number of root tips.

Induced Fe reduction segregated among the cultivars in the first few days after induction but converged by day 14 (Fig. 2). The small differences among the cultivars are probably of little consequence in the field. A healthy papaya plant is supported by the continual appearance of new root tips. Thus, the presence of fully induced root tips should be plentiful as long as there is no arrest in root system development.

The importance of maintaining a healthy, continually expanding root system is underscored by this study. Iron reductase activity in the 10 to 15-mm root section was greatly reduced in all four cultivars when compared with the root tips, and was negligible in the 60 to 70 mm root section (Fig. 1). Since these roots grew at a rate of ≥20 mm·d⁻¹, the duration of the site of highest Fe reductase activity for any given segment along the axis of the growing root was <24 h. Thus, any decline in root extension rate or the total number of root tips may strongly influence total Fe acquisition potential. These implications are even more important when considering this inducible reductase has shown activity for other metal cations (Welch et al., 1993).

The slightly higher Fe reductase activity observed on root tips of the aeroponics plants (Fig. 1) compared with the perlite plants (Fig. 2) may have been due to our procedures. We did not use any method of Fe scavenging from root or hardware surfaces when the culture solution was switched from plus-Fe to minus-Fe. Thus, the amount of trace Fe residue in the aeroponics system was probably less than in the perlite system because of greater surface area with the perlite substrate. As a result, Fe reduction potential may have developed more quickly in the aeroponics system, and may account for the slightly greater values from this culture system.

We have observed increased Fe-deficiency chlorosis of Solo and Formosa papaya plants growing in calcareous soils following foliage damage by tropical cyclones on Guam. A common response to canopy defoliation or pruning is a decline in root growth until the canopy recovers (Eissenstat and Duncan, 1992). Using observation windows, we have observed that defoliation leads to a rapid decline in root extension and the total number of root tips of papaya plants (Marler and Stushnoff, 1999). Results of the present study indicate that this decline in root extension following canopy damage is likely the cause of increased Fe deficiency symptoms, since reduction potential is primarily in the growing root tips.

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