Fractionation of Iron in Chlorotic and Nonchlorotic Tomato Leaf Tissue

B. D. KNEZEK and R. H. MAIER

Department of Agricultural Chemistry and Soils, University of Arizona, Tucson, Ariz.

Chlorotic and nonchlorotic tomato leaf tissues containing similar amounts of iron were subjected to chemical fractionation and chelate extraction to establish the presence of different forms of iron and to characterize the strength of iron binding in both tissues. Separate samples from each replicate tissue were analyzed for total iron concentration and iron extractable by each of four solvents followed by two aqueous solutions of chelating agents possessing increasing affinities for iron. The results indicate that extractable iron exists in different forms and that over 50% of the iron is bound in both tissues with relatively great tenacity.

CONTRADICTORY evidence has been presented about the correlation of total iron content with iron-deficiency chlorosis. Some researchers maintain that chlorosis is related to the total iron concentration in plant leaf tissue (*12*), while others claim there is no correlation (*9*). Attempts have been made to define iron-deficiency chlorosis in terms of chemical fractions or forms of iron. Oserkowsky (*9*) has shown a positive relationship between 1*N* HCl-soluble iron and chlorosis. He termed the soluble iron as active and the insoluble as residual. Bennett (*1*) has shown that the residual fraction increased as pear leaves aged. Little is known about the active and residual plant iron fractions.

This investigation was conducted to increase information about iron chlorosis in plants through chemical fractionation procedures. Total iron and fractions of iron extracted from chlorotic and nonchlorotic tomato leaf tissues at three stages of development are compared.

Materials and Methods

Tomato plants (*Lycopersicon esculentum*) variety Early Pak were grown in nutrient solutions under greenhouse conditions. Germination and growth in nutrient media for the first 14 days after planting were under laboratory conditions using the support method of Greene, Bullock, and Maier (*3*). The tomato seedlings in the greenhouse were placed in an aerated nutrient solution (modified Hoagland's No. 1) (*4*) with iron supplied as iron ethylenediamine di(*o*-hydroxyphenylacetic acid) (FeEDDHA) at a concentration of 0.01 p.p.m. of iron. Approximately 4 weeks after planting, the seedlings were thinned to a constant number and transferred to aerated 14-liter polyethylene pails. Hoagland's solution was used again with one half of the pails containing 0.05 p.p.m. of iron as FeEDDHA, while the other pails contained 1.5 p.p.m. of iron supplied as FeEDDHA. These levels of iron for tomato were previously found to produce chlorotic and nonchlorotic leaf tissues, respectively. After preparation, each nutrient solution was adjusted to a pH 6.8 with 1*N* KOH or 1*N* HNO_3 as needed. The pH value of each solution was checked twice each week, but no adjustments were made between the weekly solution changes. All solutions were prepared with deionized water which was subsequently used to maintain a constant volume in each pail throughout the week.

Two replicates per treatment were harvested 38, 52, and 66 days after planting. The leaves were rinsed in 0.001*N* HCl followed by deionized water and quick-frozen with dry ice. The quick-frozen plant material was dried in a refrigerated lyophilizer, ground in a Wiley mill through a 60-mesh stainless steel screen, mixed, and stored in polyethylene containers at −18° C. prior to analysis.

Each of four solvents (deionized water, methanol, ethyl acetate, and carbon tetrachloride) was used in the homogenization of an aliquot of each leaf tissue. A hand-operated glass homogenizer was used to homogenize the leaf tissue by passing a Teflon pestle through the tissue in 30 ml. of cold (4° C.) solvent. After homogenization, each homogenate was separated into filtrate and residue by Millipore filtration through a 1.5-micron solvent-resistant filter. Each residue was successively extracted with 1×10^{-3} *M* acetate buffered (pH 5.85) solutions of two chelating agents, sodium hydroxyethylethylenediaminetriacetate (NaHEEDTA) and sodium ethylenediamine di(*o*-hydroxyphenylacetate) (NaEDDHA), for 12 hours. Following 12 hours of extraction on an Omnishaker (Buchler Instruments, New York, N. Y.) at 4° C. for each chelate, the solution was separated from the residue by use of a Millipore filter. Iron was determined by an *o*-phenanthroline method (*5*) following wet oxidation with nitric and perchloric acid. Total iron per sample as well as iron content in each filtrate was determined. Several iron-extracted residues from each step in the extraction were analyzed to confirm complete iron recovery.

Results

Table I indicates that dry weights were greater for nonchlorotic than for chlorotic leaf tissues at all three harvest times. The mean total concentrations and amounts of iron in the leaf tissues are given in Table II. The concentration of iron in both chlorotic and nonchlorotic leaf tissues was approximately the same at each of the harvest times until the 66-day harvest. (Subsequent experiments have shown that the total iron concentration value for nonchlorotic leaf tissue of the third harvest time is questionable and normally should be about the same as the value for iron concentration of chlorotic leaf tissue.) Less total iron was accumulated by chlorotic than nonchlorotic leaf tissue at each harvest time. The oven-dry weights and total iron contents of stem tissues are given in Table III.

Figure 1 shows that more iron was extracted by deionized water from non-

Table I. Average Oven-Dry Weights of Tomato Leaf Tissues Produced

Harvest Time, Days	Chlorotic, G.	Nonchlorotic, G.
38	4.9	7.1
52	18.9	27.9
66	27.5	48.7

Table II. Total Concentration and Amount of Iron Present in Tomato Leaf Tissues

Harvest Time,	Chlorotic		Nonchlorotic	
Days	μg./g.	μg.	μg./g.	μg.
38	113.7	557.1	108.3	768.9
52	125.1	2364.4	187.7	5236.8
66	239.7	2591.7	167.3	8147.5

Table III. Oven-Dry Weights and Iron Content of Tomato Stem Tissues

	Chlorotic			Nonchlorotic		
Harvest Time, Days	G.	μg./g.	μg.	G.	μg./g.	μg.
38	4.4	34.8	153.1	5.6	82.7	463.1
52	22.9	822.4	513.0	25.1	42.6	1069.3
66	37.2	43.0	1599.6	74.1	50.5	3742.0

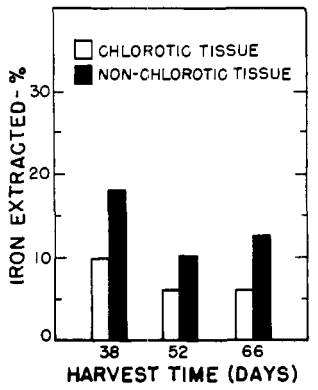

Figure 1. Iron extracted by deionized water from chlorotic and nonchlorotic tomato leaf tissues harvested at three successive times after planting

chlorotic than from chlorotic leaf tissues at each stage of growth. Solvents other than deionized water extracted negligible amounts of iron. Figure 2 gives the percentages of iron extracted by HEEDTA [*N*-carboxymethyl)-*N'*-(2-hydroxyethyl) - *N,N'* - ethylenediglycine] from solvent-extracted leaf tissues of the earliest to latest stage of growth, respectively. More iron was removed from the nonchlorotic than chlorotic leaf tissues in each instance.

The percentages of iron removed by EDDHA {*N,N'*-ethylenebis[2-(*o*-hydroxylphenyl) glycine]} from the solvent and HEEDTA-extracted leaf tissues are shown in Figure 3; the removal was not consistent from nonchlorotic as compared to chlorotic tissues at any stage of growth. The greater total cumulative percentages of iron extracted by each solvent and the two chelating agents from nonchlorotic as compared to chlorotic leaf tissues are given in Figure 4 for earliest to latest stage of growth, respectively.

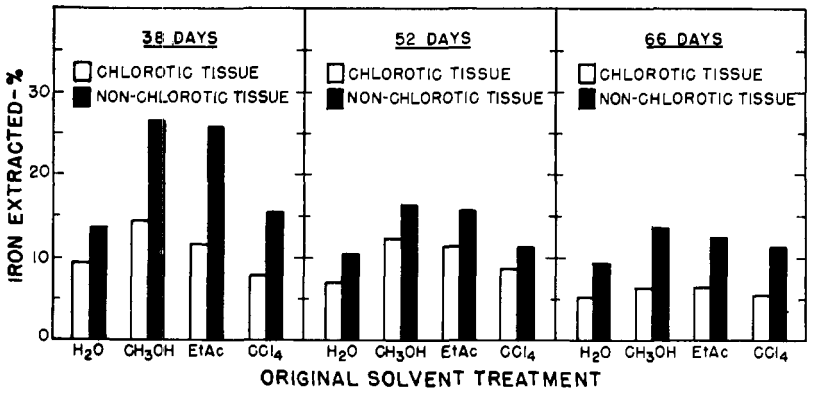

Figure 2. Percentages of iron removed by HEEDTA from solvent-extracted chlorotic and nonchlorotic tomato leaf tissues harvested at three successive times after planting

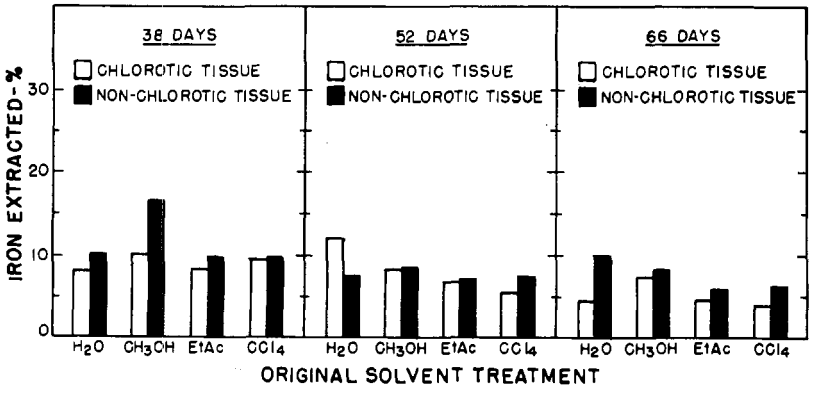

Figure 3. Percentages of iron removed by EDDHA from solvent- and HEEDTA-extracted chlorotic and nonchlorotic tomato leaf tissues harvested at three successive times after planting

Discussion

Table I shows that the production of new growth was impaired during the induction of iron chlorosis and that plants grown at the 0.05-p.p.m. level of iron produced less total material than plants grown at the 1.5-p.p.m. level. Data in Table II establish that the total iron concentration of the leaf tissues is not necessarily correlated with the presence or absence of iron-deficiency chlorosis. Jacobson and Oertli (*6*) have reported that once chlorosis is induced because of a lack of sufficient iron at an early stage of growth, the chlorosis persists, even though the same tissues may accumulate iron later. A similar effect may have occurred in the tomato leaf tissue (Table II). These observations indicate that the form of iron in the tissues may be important in iron-deficiency chlorosis.

Figure 1 shows a difference in the percentage of deionized water–extractable iron from chlorotic as compared to nonchlorotic leaf tissues at all three stages of growth. Similar results have been reported for copper (*8*). These data, in agreement with earlier findings (*2*), indicate that different torms of iron are water-extractable from nonchlorotic compared to chlorotic tomato leaf tissues.

The data given in Figure 2 reveal that an aqueous buffered solution of HEEDTA removed different percentages of iron from chlorotic and nonchlorotic leaf tissues regardless of the previous solvent treatment or the stage of growth. The extraction pattern in Figure 2 was due to the presence of the chelate, since the same extraction procedure, but with no chelating agent, did not yield additional iron. Different fractions of iron are removed from nonchlorotic and chlorotic tomato leaf tissues by an extracting agent with an affinity for iron slightly greater than the affinities for iron of several plant metabolites (*10, 11*).

The data represented in Figure 3 indicate that approximately equal percentages of iron are removed by EDDHA extraction from the solvent- and HEEDTA-extracted tissues. Apparently, the forms of iron removed by EDDHA are the same for nonchlorotic and chlorotic tomato leaf tissues regardless of previous extraction treatment or stage of plant growth.

The data in Figure 4 summarize the evidence for a greater cumulative percentage of extractable iron from nonchlorotic than chlorotic tomato leaf tissues at all three stages of growth. Over 50% of the iron remained in the leaf tissues after the three successive extractions. Recent research from our laboratory, with differential centrifugation of tomato leaf cell particulates, shows that much of the iron in the plant cell might have structural functions (*7*). This can-

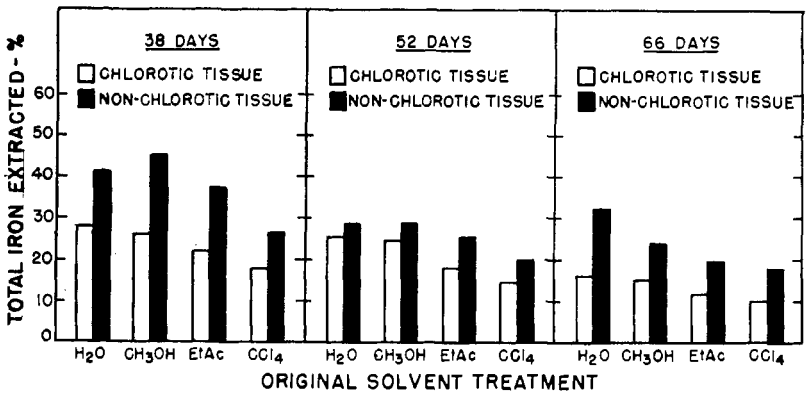

Figure 4. Total percentages of iron extracted by HEEDTA and EDDHA from chlorotic and nonchlorotic tomato leaf tissues harvested at three successive times after planting

not be ignored, since much of the iron is relatively resistant to removal and may reside in the wall of the cell.

Similar fractionations of iron in stem and root tissues provided little clue as to the forms of iron in chlorotic as compared with nonchlorotic tissue because of inconsistent data.

Literature Cited

(1) Bennett, J. P., *Soil Sci.* **60,** 91 (1945).
(2) Bowen, H. J. M., Cawse, P. A., Thick, J., *J. Exptl. Botany* **13,** 257 (1962).
(3) Greene, R. E., Bullock, J. S., Maier, R. H., *Agron. J.* **54,** 363 (1962).
(4) Hoagland, D. R., Arnon, D. I., "Water-Culture Method for Growing Plants without Soil," Calif. Agr. Expt. Sta. Circ. **347** (revised) (1951).
(5) Jackson, M. L., "Soil Chemical Analysis," Prentice-Hall, Englewood Cliffs, N. J., 1960.
(6) Jacobson, L., Oertli, J. J., *Plant Physiol.* **31,** 199 (1956).
(7) Maier, R. H., Cattani, R. A., *Life Sciences* **4,** 391 (1965).
(8) Mills, C. F., *Biochem. J.* **57,** 603 (1954).
(9) Oserkowsky, J., *Plant Physiol.* **8,** 449 (1933).
(10) Schmid, W. E., Gerloff, G. C., *Ibid.*, **36,** 226 (1961).
(11) Tiffin, L. O., Brown, J. C., *Science* **135,** 311 (1962).
(12) Wallihan, E. F., *Am. J. Botany* **42,** 101 (1955).

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MINERAL NUTRITION IN PLANTS

Chemical Fractions of Plant Potassium, Calcium, and Magnesium as Influenced by Soil Treatment

R. H. MAIER and B. D. KNEZEK

Department of Agricultural Chemistry and Soils, University of Arizona, Tucson, Ariz.

Total, water- and acid-soluble potassium, calcium, and magnesium have been studied in tomato stem and leaf tissue as a function of soil applications of potassium and magnesium. Total leaf potassium was highest as a result of potassium and magnesium applications. Highest amounts of water-soluble plant magnesium resulted from the high rates of potassium and magnesium applications. Potassium applications did not inhibit plant uptake of magnesium. Approximately 95% of both stem and leaf potassium was water and acid soluble. More stem and leaf calcium was acid soluble than was water soluble. However, more than half of the stem and leaf calcium was not soluble in water or acid. Proportionately more plant magnesium was extracted with acid than with water.

In an effort to understand better mineral nutrition of plants with regard to potassium, calcium, and magnesium, it would be helpful to obtain further information on the forms of these elements in certain plant parts. This approach is not only essential for the plant scientist, but information is now available indicating that plant forms of elements strongly influence animal utilization of the element ingested (*5, 11*).

The total amount of an element in a tissue is not a reliable criterion to evaluate its relation to growth and function. Elucidation of the active form (*4*) of the nutrient should be a continual goal. A prerequisite to research on the available or active forms of potassium, calcium, and magnesium in plants is predicated on a knowledge of the forms of occurrence of the element within selected tissues, and how that form changes as a function of environmental components.

The purpose of this investigation was to contribute information on the total, water- and acid-soluble amounts of potassium, calcium, and magnesium in tomato stem and leaf tissue as a function of soil applications of potassium and magnesium. Previous data on total amounts of potassium, calcium, and magnesium in tomato plants have been compiled (*1, 9*).

Materials and Methods

Materials. Laveen loam, 0 to 12 inches, was obtained from the Mesa Experimental Farm, Mesa, Ariz. After collection, the soil was air-dried, crushed with a wooden rolling pin, and sieved through a plastic screen with 10-mm. openings.

The inside surfaces of No. 10 tin cans were coated with asphalt emulsion, and each treatment described below was replicated three times. The necessary amount of soil for each can was placed in a twin-shell plastic blender, and the appropriate nutrients were added. The mixture was blended for 20 minutes and then was transferred to the respective container.

Base Nutrient Applications. The base nutrient application for all treat-