Intracellular Distribution of Iron, Catalase, and Protein in Tomato Plant Tissue

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Iron concentration, catalase activity, and protein concentration have been studied in the particulate fractions of leaf, stem, and root tissues of chlorotic and nonchlorotic tomato plants. Iron accumulated early in the tissues. The root tissue had the highest iron content. The iron concentration of the fractions of all tissues was centered in the plastids-nuclei and mitochondrial fractions, but the majority of the total iron of the tissue cells was located in the cell debris fraction. The iron content of the leaves may be correlated with chlorosis, but this relationship in the stem is not as definite. The supernatant was the major protein-containing fraction, and the mitochondrial fraction was highest in catalase activity. No definite relationship between chlorosis and a lower catalase activity is indicated.

Chlorosis in plants has been observed for many years. The complete function of iron in the chlorotic plant has never been determined fully, but much speculation has been offered as to why chlorosis occurs (*1*, *5*, *6*, *9*, *13*, *20*, *29*). In much of the previous work, the entire tissue, such as the leaf or the stem, has been analyzed for iron content. The numerous cells in these tissues are composed of distinct particulates which carry out the various metabolic reactions, and, hence, a knowledge of the intracellular distribution of iron may be of value in determining further the function of iron and its relation to chlorosis and growth.

Cell particulates may be separated by differential centrifugation. Some investigators dismiss this procedure on the grounds that cell rupture necessarily produces artifacts such as redistribution, adsorption, and other alterations. If precautionary steps, such as use of isotonic buffer solutions, decreased temperature, careful manipulation and washing and thorough microscopic examination, are taken in an attempt to counteract these limitations, then differential centrifugation is a valuable technique.

Chlorosis in many cases does not depend strictly upon the iron content of the plant (*12*, *14*, *19*), and therefore, some investigators have tried to relate enzymatic activities to this abnormality (*2*, *3*, *7–10*, *13*). Consequently, catalase, a widespread, iron-containing enzyme, has been used as a tool in the study of chlorosis (*4*, *7–10*) and has decreased activity under chlorotic conditions.

In this investigation, iron content and catalase activity were measured in the particulate fractions of chlorotic and nonchlorotic tomato leaf, stem, and root tissue. Protein determinations were also made in order to observe the effects of chlorosis on protein content and to ascertain if iron and catalase, expressed in terms of protein content, would provide a better correlation with chlorosis than either by itself.

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Materials and Methods

Greenhouse. Tomato plants (*Lycopersicon esculentum* Mill.), variety Early Pak, were grown in the greenhouse in iron sufficient and deficient nutrient solution cultures using polyethylene beads as a supporting medium (*15*). The nutrient solution was prepared according to Hoagland and Arnon (*18*) for solution No. 1 with the exception that iron was applied as Fe Chel 138 [ferric chelate of ethylenediamine di(*o*-hydroxyphenylacetic acid); Geigy Chemical Corp., Yonkers, N. Y.] Iron levels of 0.05 and 1.0 p.p.m. were suitable for obtaining chlorotic and nonchlorotic tomato plants, respectively. Plants were harvested 28 and 49 days after planting. Two replicates from each treatment were harvested. The plants were divided into leaves, stems, and roots, and washed once with 0.01*N* HCl and twice with deionized water. The washed tissue was blotted dry, immediately frozen, and then dried under vacuum in a mechanically refrigerated lyophilizer. After lyophilization, the plant tissue was weighed and then ground in a Wiley mill to pass a 60-mesh stainless steel screen. The ground tissue was stored at −18° C. until needed.

Homogenization. The ground tomato tissue—approximately 1 gram—was homogenized in 20 ml. of a 0.2*M* Na_2HPO_4–0.2*M* NaH_2PO_4–0.3*M* sucrose buffer, pH value of 7.0. The tissue was homogenized with a Vir Tis 45 homogenizer for 10 minutes at three-fourths speed. Preliminary microscopic studies revealed that these conditions produced approximately 85% cell breakage. Homogenization was conducted at a constant temperature of 4° C.

Fractionation. After being homogenized, the tomato tissue was separated by differential centrifugation into four fractions: cell debris (100 × G), plastids-nuclei

(1500 × G), mitochondrial-sized particles (25,000 × G), and supernatant. Each centrifugation was for 10 minutes. The nomenclature denotes which particles or particulates were present in major amounts as determined by staining and microscopic examination. The pellet of each fraction was washed three times with deionized water, and the washings were added to the supernatant of that particular fraction. The pellet of each fraction was resuspended and made up to a constant volume as shown in Table I. Aliquots were taken from these volumes for each of the determinations shown in Table I.

Catalase. Catalase activity was measured manometrically. The reaction vessel contained 1.0 ml. of a 0.2*M* NaH_2PO_4–0.2*M* Na_2HPO_4 buffer, pH 7, and 2.0 ml. of tissue sample. The side arm contained 1.0 ml. of 0.12% H_2O_2. The contents of the flask were equilibrated in a water bath, 15° C., for 30 minutes before tipping in the H_2O_2. Readings were made at 2, 4, and 10 minutes. In nearly every case, the maximum rate of catalase activity was occurring at the 4-minute point in the reaction. The catalase activity expressed in this study is this observed maximum rate.

Protein. Protein was determined by the method of Lowry and coworkers (*24*) with slight modifications. The tissue samples except the supernatant fraction (Table I) were extracted with NaOH—final concentration 3.0*N*—for 20 hours at 4° C. The extracts were removed by centrifugation and diluted to the volume shown in Table I. Trichloroacetic acid (TCA, 20%) was used to precipitate protein—TCA-insoluble nitrogen compounds. The precipitate was obtained by centrifugation at 10,000 × G for 10 minutes and washed with 5% TCA. The precipitate was then dissolved with 0.3 ml. of 1.0*N* NaOH and the protein determined using Folin's reagent. A working curve for determining protein content was prepared from a crystalline bovine albumin standard solution.

Iron and Dry Weight. Iron determinations and dry weight measurements were made on the sample from the remaining pellet volume (Table I). The samples were dried, weighed, and then oxidized using perchloric acid. The residue was taken up with 0.1*N* HNO_3 and placed in 25-ml. volumetric flasks along with 10 ml. of acetate buffer, pH 4.5, 1.0 ml. of 10% hydroxylamine hydrochloride, 3.0 ml. of 0.2% 1,10-phenanthroline, and sufficient deionized water to bring the contents to volume (*21*). The transmittances were read at a wave length of 500 mμ with a Bausch and Lomb Spectronic 20 spectrophotometer. The supernatants were read after 24 hours owing to a hindrance of rapid-color development by phosphates (*17*). Iron content was determined from a standard curve.

Results

The root tissue is compared only in a relative manner in this study because only the second harvest provided sufficient material to analyze. The data show that the leaves had a higher iron concentration (Table II) and greater total protein (Table III) than did the stems, but the catalase activity of the two tissues was not greatly different (Table III). The iron concentration of the root at the second harvest was higher than that of either the leaves or stems.

The iron content of the tissues was higher at the second harvest than at the first, but the iron concentration was lower at the second harvest. The concentration of iron in the nonchlorotic tissue was higher than that in the respective chlorotic tissue at both harvests (Table II). The protein concentration of chlorotic leaf tissues decreased from the first to the second harvest whereas the protein concentration of nonchlorotic leaf tissue followed an opposite trend (Table III). The catalase activity was depressed in both leaves and stems at the second harvest compared with the first harvest. The chlorotic leaf tissue showed a slightly lower catalase activity than the nonchlorotic leaf tissue (Table III).

In the particulate fractions, the plastids-nuclei and mitochondrial fractions contained the highest concen-

Table I. Fraction Volumes and Aliquots Used in Milliliters

Fraction[a]	Final Vol.	Catalase	Protein: Vol. for NaOH extract	Protein: Final vol.	Protein: Vol. of extract used	Iron and Dry Wt.
CD	50	2	10	50	0.5	36
P-N	25	2	5	25	0.5	16
M	50	2	10	50	0.5	36
S	250	2	...	...	0.25	100

[a] CD = Cell debris; P-N = Plastids-nuclei; M = Mitochondria; S = Supernatant.

Table II. Dry Weight, Iron Content and, Concentration of Tomato Leaf, Stem, and Root Tissue at Two Levels of Iron and at Two Periods of Growth[a]

Iron Level, P.P.M.	Anatomical Part	Dry Wt., Grams	Iron Concn., μg. per Gram	Iron Content, μg.
	Growth Period—28 Days			
	Leaf	3.9	71.8	279.3
0.05	Stem	2.9	29.8	86.0
	Root	0.9	...	...
	Leaf	6.4	154.5	985.1
1.0	Stem	5.1	43.8	222.7
	Root	2.9	...	...
	Growth Period—49 Days			
	Leaf	11.4	61.7	703.4
0.05	Stem	14.0	19.7	275.4
	Root	2.0	68.8	134.1
	Leaf	11.4	117.6	1334.5
1.0	Stem	13.1	23.9	313.2
	Root	2.8	179.7	508.6

[a] Data represent means of two replications.

Table III. Distribution of Iron, Protein, and Catalase Activity[a] in Tomato Tissue Particulate Fractions

Iron Level, P.P.M.	Parameter	Leaf							
		CD[b]	P-N	M	S	CD	P-N	M	S
		Growth Period—28 Days				Growth Period—49 Days			
	Dry wt., grams	0.4997	0.0536	0.0704	0.3764	0.6078	0.0542	0.0518	0.2886
	Fe, μg. per gram fraction	88.8	258.0	193.0	...	72.6	173.1	158.3	...
	Fe, % distribution	61.8	19.3	18.9	...	71.5	15.2	13.3	...
0.05	Protein, mg. per gram fraction	122.3	315.7	534.8	303.5	97.5	224.4	266.8	234.4
	Protein, % distribution	26.6	7.4	16.4	49.7	38.9	8.0	9.1	44.1
	Catalase, μl. O_2 per min. per gram fraction	252.8	741.0	1,353.4	575.0	178.3	567.2	1,150.0	580.0
	Dry wt., grams	0.4822	0.0635	0.0524	0.4025	0.5591	0.0580	0.0560	0.3272
	Fe, μg. per gram fraction	207.0	392.3	568.3	...	133.0	414.7	342.7	...
	Fe, % distribution	64.6	16.1	19.3	...	63.2	20.4	16.3	...
1.0	Protein, mg. per gram fraction	80.3	228.0	295.6	154.2	109.4	328.3	383.2	246.5
	Protein, % distribution	29.6	11.1	11.8	47.5	33.6	10.4	11.8	44.2
	Catalase, μl. O_2 per min. per gram fraction	287.8	884.6	1,606.7	724.4	237.5	860.9	1,532.5	840.3

Iron Level, P.P.M.	Parameter	Stem							
		CD[b]	P-N	M	S	CD	P-N	M	S
		Growth Period—28 Days				Growth Period—49 Days			
	Dry wt., grams	0.6662	0.0293	0.0293	0.2754	0.8032	0.0308	0.0234	0.1430
	Fe, μg. per gram fraction	28.2	201.7	176.1	...	12.1	50.6	359.0	...
	Fe, % distribution	62.9	19.8	17.3	...	49.4	7.9	42.7	...
0.05	Protein, mg. per gram fraction	11.4	110.9	211.6	103.1	5.9	146.1	259.4	155.5
	Protein, % distribution	16.7	7.2	13.6	62.5	12.6	12.0	16.2	59.2
	Catalase, μl. O_2 per min. per gram fraction	74.9	810.2	1,410.5	687.1	40.8	837.7	915.1	792.3
	Dry wt., grams	0.7642	0.0358	0.0372	0.1634	0.7395	0.0271	0.0250	0.2088
	Fe, μg. per gram fraction	31.2	194.1	350.8	...	22.7	138.4	133.6	...
	Fe-% distribution	54.4	15.8	29.8	...	70.3	15.7	14.0	...
1.0	Protein, mg. per gram fraction	20.9	225.1	447.3	252.3	9.1	210.0	464.0	100.3
	Protein, % distribution	19.5	9.8	20.3	50.3	14.9	12.7	25.8	46.6
	Catalase, μl. O_2 per min. per gram fraction	50.1	633.9	857.8	790.8	42.8	650.8	999.8	493.8

[a] Maximum rate after 4 minutes of reaction. Data represent means of two replications.
[b] See Table I for abbreviations.

tration (amount of iron per gram of particulate fraction) of iron in all three tissues—the leaves, stems, and roots. As mentioned for the tissues in general, however, the concentration in the stems was much lower than that in the leaves and roots. The cell debris contained most of the iron. No iron was detected in the supernatant fraction.

During maturation several shifts in the distribution of the iron occurred (Table III). In the leaves, the chlorotic cell debris fraction increased in per cent of iron at the expense of the other fractions, whereas in the nonchlorotic tissue a shift in distribution occurred only between the plastids-nuclei and mitochondrial fractions. The cell debris weight increased under both conditions. In the stems, the chlorotic tissue underwent a radical change in its iron distribution with the mitochondrial fraction gaining greatly at the expense of the other fractions. The nonchlorotic tissue, however, showed a decrease in the mitochondrial proportion of iron with the cell debris fraction increasing. In this stem tissue, the cell debris weight increased for the chlorotic tissue, but the supernatant showed the weight increase for the nonchlorotic tissue.

The cell debris fraction of the leaves had the lowest protein concentration although it and the supernatant fraction had the highest total protein (Table III). The stem mitochondrial fraction had the highest protein concentration, but the supernatant contained the greatest total amount of protein (Table III). In both the leaf and stem, the mitochondrial fraction had the highest catalase activity. The cell debris fraction exhibited some activity.

Discussion

The lower iron concentration in the chlorotic tissue would imply that iron has an effect on reducing the apparent chlorophyll content of the plant. This has also been suggested by other authors.

The need for iron in areas of major metabolic activity necessitates the presence of greater amounts of iron in leaves than in stems, and the data bear this out.

The higher iron concentration at the first harvest as compared with the second harvest indicates that the largest accumulation of iron occurred during the early stages of growth, and that as the plant matured, this rapid uptake of iron decreased. A drop in iron accumulation coupled with continued vegetative growth would account for the decrease in concentration of iron in the tissues.

The high iron concentration in the root at the second harvest date is supported by the findings of Oborn (*27*) and McGeorge (*25*). The high values found for roots suggest that the root may satisfy its own needs first before transporting any of the iron, and since the root tissues from chlorotic plants had only about half the iron concentration as compared with the nonchlorotic plants, the authors assumed that the external iron levels did have an effect on the iron uptake by the root tissues.

That the highest iron concentration was found in the plastids-nuclei and mitochondrial fractions is reasonable in that these are the sites of photosynthesis, respiration, and other metabolic processes in which iron is known to function or is suspected to have an active part. The high iron content of the cell debris fraction is unlike the findings of some investigators (*22*, *26*, *29*), but is similar to the findings of Cattani (*11*). It is not known whether this iron is part of a compound or complex or has merely been trapped among the cell debris components. The possibility that this iron is due to contamination or adsorption to the surfaces of the particulates during the homogenization and fractionation procedures does not seem to be the case in light of the work with Fe^{59} by Cattani (*11*) which showed that iron, when added to the homogenate and allowed to equilibrate for three days, was not adsorbed by the particulates and was found almost entirely in the supernatant fraction.

The lack of detectable iron in the supernatant may be a function of the orthophenanthroline method used for measuring the iron content. More sensitive methods will have to be utilized to prove or disprove this supposition.

The change in the iron percentage of the leaf and stem fractions during maturation, along with weight changes in the fractions, seems to explain the relative differences in iron concentration between the chlorotic and nonchlorotic tissue. The significance of this iron percentage redistribution, however, is not understood at present.

The high values for protein in the supernatant fraction should be expected because of soluble protein and because ribosomes, which are active in protein synthesis are also probably part of this fraction. Elution from other fractions may be taking place, also. Protein elution, however, is in opposition to the work by Zucker and Stinson (*30*) who discovered that plastids lost little protein if isolated in a sucrose medium containing phosphate as a buffering ion.

The high catalase activity of the mitochondrial fraction is supported by the findings of other investigators (*16*, *23*, *28*). That the plastids-nuclei fraction had a lower catalase activity than the mitochondrial fraction is contrary to the findings of Neish (*26*), though, who reported that catalase is highly concentrated in the chloroplasts. Jagendorf and Wildman (*23*), however, found that purified chloroplasts of tobacco leaves contained little or no catalase activity.

Catalase activity may not necessarily be endogenous to the supernatant fraction, because the catalase in this fraction may have been eluted from the other particulates. In fact, Greenfield and Price (*16*) have shown that catalase is eluted from mitochondria by repeated washings with sucrose buffer.

The cell debris fraction exhibited slight catalase activity, but this may be due to contamination of the cell debris by trapped plastids and mitochondria. More purified cell debris fractions would be needed in order to check this possibility further.

Catalase activity did not follow the fraction relationships for iron concentration as well as it did for protein concentration (Table III). This may indicate that the iron concentration was not related to catalase activity,

but it cannot be concluded, either, that there is any definite catalase–total protein relationship.

Although catalase activity was lower in chlorotic leaves, no definite relationship between catalase activity and chlorosis can be drawn for the tissues as a whole. Also, the iron and catalase activity expressed on a per unit protein basis did not show any better correlation with chlorosis than either by itself.

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