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Influence of Iron Supply on Peroxidase Activity and Peroxidase Isozymes in Corn (*Zea mays*)¹

R. L. Ulmer, F. A. Haskins, and W. R. Akeson²

ABSTRACT

Plants of Oh43 × Wf9 corn (*Zea mays* L.), grown in a sand culture system, were watered daily with nutrient solution containing 0.025, 1.25, or 2.5 ppm Fe. Plants also were grown in a soil-sand mixture. Leaf extracts were assayed quantitatively for peroxidase activity, and samples of the extracts were subjected to gel electrophoresis. A positive relationship was observed between Fe supply and peroxidase activity. Numerous peroxidase isozymes were detected by gel electrophoresis. As judged by intensity of the bands, all isozymes appeared to be influenced by Fe supply.

A GARWALA et al. (1) and Brown and Hendricks (2) reported reduced peroxidase activity in preparations of corn (*Zea mays* L.) leaves from plants grown under low-iron conditions. Their work did not include an investigation of the isozymes of peroxidase. Other studies have shown peroxidase isozyme patterns to be influenced by such factors as growth stage, genetic constitution, and disease (4, 10). This report deals with the peroxidase isozyme patterns of corn plants differing, because of level of Fe supply, in total peroxidase activity.

MATERIALS AND METHODS

Corn (Oh43 × Wf9) was grown in the greenhouse in a free-drainage sand culture system. Clay pots (16 cm in diameter) were lined with plastic and filled with white silica sand that had been washed with 2 N HCl and thoroughly rinsed with distilled water. Kernels were planted at the rate of four per pot, and plants were thinned, about 2 weeks after planting, to one per pot in Experiment 1 and two per pot in Experiment 2. In both experiments the sand was watered daily with distilled water during the 1st week after planting. Thereafter, the sand was saturated daily with nutrient solution. Except for concentrations of Zn and Fe, the nutrient solution was identical to 0.25-strength Hoagland and Arnon Solution I (6). The zinc concentration was 1.0 ppm; Fe levels were varied as indicated in Table 1. The source of iron was Geigy's Sequestrene 330 Fe (monosodium ferric diethylenetriaminepentaacetate or NaFeDPTA). Other chemicals used in the solution were reagent grade.

For purposes of comparison, plants grown in a soil-sand mixture (approximately 60% greenhouse soil and 40% sand) were included in the study. These plants, referred to as "Controls," received distilled water only, except for several applications of 0.00375 M KNO₃, made on successive days when the plants were about 4 weeks old, to guard against possible N deficiency.

At the time of sampling, plant height was measured from the surface of the sand or soil to the tip of the longest leaf. Fresh weight determinations included only the aboveground portions of the plants. Samples used for protein and enzyme

assays consisted of leaf tissue (Table 1) with the midrib and tip removed. In a coldroom (2 C) these samples were ground in a mortar with a little sand and 10 ml of buffer (0.1 M tris, 0.5 M sucrose, pH 8.0)/g of fresh tissue. These crude preparations were centrifuged (39,000 g, 30 min, 2 C), and supernatant fractions were used as enzyme preparations. Preliminary experiments indicated that these fractions contained more than 90% of the peroxidase activity of the crude preparations. The procedure of Lowry et al. (7) was used to determine the concentration of trichloroacetic acid-precipitable protein in the enzyme preparations of Experiment 1, and Potty's modification (9) of the Lowry procedure was used in Experiment 2.

Peroxidase activity was assayed by a modification of the spectrophotometric procedure described by Haskins (5). The reaction mixture consisted of 2.65 ml of 0.01 M sodium acetate buffer, pH 5.6; 0.70 ml of 0.18 M guaiacol; 0.10 ml of 0.145 M hydrogen peroxide; and 0.05 ml of diluted enzyme preparation. On the basis of preliminary trials, dilutions of enzyme preparations were adjusted to provide approximately equal rates of absorbance change in the various reaction mixtures. Each preparation was assayed in duplicate. Buffer and guaiacol solutions were brought to 30 C before reaction constituents were mixed; hydrogen peroxide and enzyme preparations were held in ice until used.

A modification of the procedure of Davis (3) was used for gel electrophoresis. A 7% acrylamide running gel was employed. The gel buffer was 0.378 M tris, pH 8.9. Spacer and sample gels were not used; rather, the enzyme preparation was applied in the extraction buffer directly to the surface of the running gel. The chamber buffer was 0.0124 M tris, 0.096 M glycine, adjusted to pH 8.3 with HCl. The power supply provided constant voltage (180 volts), and current was varied from an initial value of about 2.7 milliamperes/gel to about 2.0 milliamperes/gel at the end of the run. Duration of electrophoresis was about 90 min. All electrophoretic runs were done in a coldroom (2 C).

After electrophoresis gels were incubated with guaiacol or benzidine-HCl to detect bands having peroxidase activity (4). When guaiacol was used as the substrate, gels were immersed for 30 min at room temperature in 0.018 M guaiacol, rinsed twice with deionized water, and then immersed in 0.015% hydrogen peroxide-1% acetic acid. Bands developed in about 10 min. With benzidine-HCl (8) as the substrate, gels were immersed in a mixture of 0.02% benzidine-HCl, 1% acetic acid, and 0.015% hydrogen peroxide. An immersion time of 3 to 5 min was used for distinguishing between slow-migrating bands, and approximately 15 min was required for development of the more mobile but less intense bands. After staining, gels were transferred to a fixing solution consisting of methanol, water, and glacial acetic acid (5:5:1, v/v/v).

RESULTS AND DISCUSSION

At the time of sampling, plants had 7 to 9 visible leaves. The three oldest leaves on all plants in both

Table 1. Description of treatments and samples. See text for further details.

Exp.	Treatment, ppm iron	Plant age at sampling, days	Samples per treatment	Plants per sample	Leaves comprising sample*
1	0.025 (low)	43	3	1	1, 2, 3, 4
	1.25 (medium)	43	3	1	1, 2, 3, 4
	2.50 (high)	43	3	1	1, 2, 3, 4
	Control	37	3	1	1, 2, 3, 4
2	0.025 (low)	37	3	2	1, 2, 3, 4
	1.25 (medium)	37	3	2	2, 3, 4, 5
	2.50 (high)	37	3	2	2, 3, 4, 5
	Control	37	3	2	2, 3, 4, 5

* Leaf no. 1 was considered to be the uppermost leaf on the plant. In Experiment 2 only even numbered leaves were taken from one plant and only odd numbered from the other. Thus, each sample consisted of four leaves.

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Table 2. Influence of Fe supply on fresh weight, plant height, and soluble protein. Within experiments means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test (11).

Exp.	Mean fresh weight per pot, g			
	Iron level			Control
	Low	Medium	High	
1	7.38 c	18.47 bc	44.70 a	21.05 b
2	14.34 b	38.82 a	33.56 a	28.81 a
Exp.	Mean plant height, cm			
	Low	Medium	High	Control
1	53.3 c	74.5 b	98.1 a	98.3 a
2	59.7 c	78.0 b	90.1 a	96.0 a
Exp.	Protein content of enzyme preparation, mg/ml			
	Low	Medium	High	Control
1	1.38 b	1.65 b	2.35 a	1.55 b
2	1.27 b	1.78 a	1.93 a	1.46 b

Table 3. Influence of Fe supply on peroxidase activity. Peroxidase activity is expressed as Δ absorbance $\cdot$ min⁻¹ $\cdot$ mg protein⁻¹. Within experiments means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test (11).

Exp.	Determinations	Mean peroxidase activity			
		Iron level			Control
		Low	Medium	High	
1	6*	1.90 c	2.84 bc	3.75 b	6.21 a
2	6*	1.83 c	4.48 b	5.88 a	6.29 a

* Duplicate determinations on each of three preparations for each treatment.

experiments were green, while the younger leaves displayed varying degrees of chlorosis, depending on the treatment imposed. In the low-Fe treatment the fourth oldest leaf was somewhat chlorotic, and the degree of chlorosis increased in the younger leaves. Leaf 1 (the uppermost leaf) was completely chlorotic. Thus, in the low-Fe treatment all sampled leaves (Table 1) were more or less chlorotic. The plants given a medium level of Fe in Experiment 1 displayed about the same degree of chlorosis as the low-Fe plants, but the medium-level plants in Experiment 2 had only a green and yellow striping. The plants supplied a high level of Fe displayed a very slight chlorosis in leaves 1 and 2. Control (soil-grown) plants were free of apparent chlorosis.

Iron supply greatly affected the fresh weight and height of the plants (Table 2). Protein content, expressed as mg of protein per ml of enzyme preparation, may be taken as a measure of the soluble protein in 100 mg of fresh leaf tissue. This content appeared to increase with increasing Fe supply (Table 2).

Peroxidase activity increased with increasing Fe supply (Table 3). This trend also was observed in several preliminary experiments. This positive relationship between Fe supply and peroxidase activity agrees with previously cited observations (1, 2).

Following electrophoresis, numerous bands were observed in gels incubated with peroxidase substrates, and the differences in staining intensity were in general agreement with differences found in the activity assays. No difference in the number of peroxidase bands was observed, unless the difference in total peroxidase activity was at least 2-fold. Differences of this magnitude were observed in Experiment 2 between the low-Fe treatment and the high-Fe and control treatments. Photographs of gels from this experiment are shown in Fig. 1. The most readily apparent differences among these gels are those between the low-Fe treatment and the other treatments. In the low-Fe gels three bands of intermediate mobility, rather than four, are visible, and the intensity of these bands is greatly reduced. The possibility exists, of course, that a longer incubation time might have revealed the fourth band of intermediate mobility in the low-Fe gels.

The gels shown in Fig. 1 were immersed in the benzidine-HCl substrate for 15 min, a procedure that caused the slow-migrating bands, of which there are at least two, to appear as a single broad band. The separation of these slow-migrating bands, and the positive relationship of their intensities to the level of Fe supply, were apparent in other gels in which guaiacol was used as the substrate or in which immersion time in benzidine-HCl was only 3 to 5 min.

The foregoing results support the conclusion that the decrease in peroxidase activity associated with a decreased Fe supply cannot be ascribed to the loss of any particular isozyme(s) of peroxidase. Rather, all isozymes appeared to be reduced in quantity under conditions of low Fe supply.

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Fig. 1. Benzidine-HCl-stained (15-min immersion time) peroxidase isozymes from the low-Fe, medium-Fe, high-Fe and control treatments of Experiment 2. Samples (180 μ g protein/gel) were applied to the upper end of the gels.

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