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Cell Wall and Protoplast Isoperoxidases of Corn Leaves in Relation to Cut Injury and Infection with *Helminthosporium maydis*

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ABSTRACT

Young leaves of two corn (*Zea mays*) inbreds with normal and Texas male-sterile cytoplasm, which differed in their susceptibility to *Helminthosporium maydis* Nisikado and Miyake race T, showed no significant qualitative or quantitative differences in their isoperoxidase patterns. Of six cathodic and four anodic isoenzymes present in the soluble fraction, four and two, respectively, comprised the fraction ionically bound to the cell wall. Peroxidase fractions ionically and covalently bound to the wall constituted about 20% of the total peroxidase activity. No new isoperoxidases were detected in either inbred line in response to cutting, infection, or detachment only and exposure to darkness for 40 hours. Three isoperoxidases, all cathodic, mainly reacted to cutting injury as well as fungal infection. One of the isoperoxidases appeared responsible for the increase in the peroxidase activity of the soluble fraction while the other two were responsible for the increase in that of the fraction ionically bound to the walls. The relative increase in the latter fraction was greater for infected leaves than for mechanically injured ones. No significant differences were found between the two inbreds in their peroxidase reactions to cutting injury or infection. Thus, the corn leaf isoperoxidases were distinctive in their distribution in the cell and in their reaction to injury. Changes in their activity induced by infection may result from a nonspecific response to injury.

The nature of the involvement of peroxidases in plant resistance to fungal, bacterial, and viral diseases has been extensively investigated (7, 13, 18, 22, 28, 30). However, the emphasis has been on soluble peroxidases, separated from tissue homogenates by centrifugation, and the cell wall-bound components have usually been disregarded. Since parasitic microorganisms are in intimate association with host cell walls at entry, at the establishment of the host-parasite relationship, and during spread in the host tissues, cell wall peroxidase fractions may be specifically involved in the plant response to the parasite.

In previously reported research on tobacco pith and sweet potato root parenchyma (5), significant differences were found between cell wall and protoplast peroxidases in their isoenzyme patterns, activity, and reaction to mechanical injury. It has been shown recently that the reaction of tobacco leaf peroxidase to cutting injury did not differ from that to tobacco mosaic virus infection in respect to the qualitative isoperoxidase patterns in the protoplast and cell wall-free or ionically and covalently bound fractions (4).

This report deals with the reaction of corn leaf peroxidase to cutting injury and to infection with the fungus *Helminthosporium maydis*. Funk inbred lines with normal and Texas male-sterile cytoplasm were used. In a previous investigation (10), the Tms[1] cytoplasm inbred showed greater susceptibility to the fungus than did the N cytoplasm inbred; this was also reflected in a greater electrolyte and peroxidase leakage from the infected leaves.

MATERIALS AND METHODS

The plants were grown in vermiculite on a 4-fold diluted Hoagland's solution in a growth chamber at 28 C and 16-hr daily photoperiod (1200 ft-c). At the four leaf stage, the laminae of the second and third leaves were detached and immediately weighed, stapled to a sheet of Whatman No. 3 filter paper, and cut into 8-mm segments or sprayed with a *H. maydis* race T spore suspension (20,000–30,000/ml) in H_2O containing 0.1% Tween 40. The suspension was prepared from cultures incubated on a glucose-L-asparagine agar medium for 12 to 14 days at 28 C. Intact (control) and cut laminae were sprayed with water containing 0.1% Tween 40. Six laminae from three plants were used in each of three replications per treatment. The laminae were incubated in the dark in a water vapor-saturated atmosphere for 16 and 40 hr. At the end of incubation, the infected laminae of the N and Tms cytoplasm inbreds had oblong lesions 1 to 2 and 3 to 4 mm long, respectively. The Tms inbred showed also slight chlorosis and some flaccidity.

Immediately after sampling, the tissue was frozen and kept in a deep freeze. Unfortunately, attempts to isolate free peroxidase from cell walls before freezing by vacuum-infiltration, followed by centrifugation, were not successful. Therefore, the protoplast peroxidase fraction was separated together with the free fraction in the cell wall.

Procedures related to peroxidase isolation were similar to those previously used (5). Triton X-100 extracts contained trace or no peroxidase activity; the wall debris was treated twice with 1 M NaCl in order to isolate the ionically bound fraction. Peroxidase determination, electrophoresis, and iso-

[1] Abbreviations: Tms: Texas male-sterile; N: normal.

peroxidase scanning were carried out as previously (3). No differences were found in the number or mobility of isoperoxidases revealed by benzidine and guaiacol. The latter was used as the hydrogen donor in the experiments as in (3). Since extracts from control tissue were present during each electrophoretic run, the relative activities of isoperoxidases in various treatments could be converted into absorbance values in relation to total peroxidase activity in the control, determined before electrophoresis.

RESULTS

Immediately after detachment, the peroxidase activities in the laminae of both inbreds were similar (Table I). The soluble fraction and that ionically bound to the walls constituted 80 and 12 to 15% of the total cell peroxidase activity, respectively. No differences were found between the inbreds in the qualitative isoperoxidase patterns. Starch gel electrophoresis revealed the presence of six cathodic and four anodic isoenzymes in the soluble fraction (Fig. 1). In both inbreds, A2 was the dominant isoperoxidase and C5 and C6 were the minor ones (Table II).

Table I. *Effects of Cutting Injury and Infection with H. maydis Race T on Peroxidase Activity in Leaf Laminae of Tms and N Cytoplasm Corn Inbreds*

Peroxidase	Laminae						
	Immediately after detachment	16 hr after treatment			40 hr after treatment		
	Control	Control	Cut injured	Infected	Control	Cut injured	Infected
	ΔA/min·g fresh wt						
Tms cytoplasm inbred							
Protoplast soluble	250	258	450	383	329	440	385
Cell wall soluble							
Free							
Ionically bound	37	50	61	54	61	90	159
Covalently bound	13	14	21	16	21	22	27
Total	300	322	532	453	411	552	571
N cytoplasm inbred							
Protoplast soluble	221	251	439	373	321	464	429
Cell wall soluble							
Free							
Ionically bound	44	54	64	66	68	110	154
Covalently bound	18	17	18	16	25	26	25
Total	283	322	521	455	414	600	608

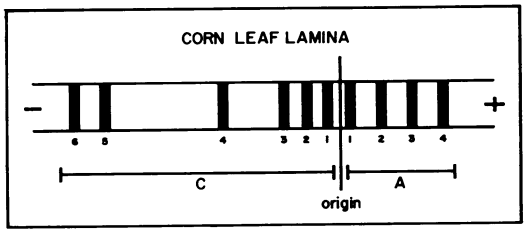

FIG. 1. Isoperoxidase pattern in leaf lamina of Funk corn inbred with either normal cytoplasm or Texas male sterile cytoplasm. Starch gel electrophoresis at pH 8.3, 10 v per cm for 3 to 4 hr. A: anodic; C: cathodic isoenzymes revealed using guaiacol-H_2O_2 as substrates.

Table II. *Effects of Cut Injury and Infection with H. maydis Race T on Activity of Protoplast and Ionically Bound Wall Isoperoxidases in Leaf Laminae of Tms Cytoplasm Corn Inbreds*

Effects observed in the N cytoplasm inbred were similar to those found in the Tms cytoplasm inbred.

Isoperoxidases	Immediately after Detachment		40 Hr after Detachment					
	Control		Control		Cut injured		Infected	
	Protoplast[1]	Wall	Protoplast	Wall	Protoplast	Wall	Protoplast	Wall
	ΔA/min·g fresh wt							
Cathodic								
C6	1	2	3	4	6	7	4	10
C5	2	11	5	14	19	49	5	81
C4	13		28		39		40	
C3	38		63		115		94	
C2	20	4	23	12	35	13	28	23
C1	35	13	36	16	37	16	39	17
Anodic								
A1	31	6	30	8	34	9	33	10
A2	76	1	91	2	92	2	93	2
A3	20		24		25		21	
A4	14		17		17		18	
Total	250	37	320	56	419	96	375	143

[1] Protoplast includes the free wall fraction.

The ionically bound fraction in the walls consisted only of four cathodic and two anodic isoperoxidases, detected also in the soluble fraction; C1 and C5 amounted to 65 to 75% of the total activity of the ionically bound fraction in both inbreds.

Even though the cell walls were treated twice with the cellulase-pectinase solution, about 80% of the total covalently bound fraction still remained in the wall debris. Therefore, no clear picture of the isoperoxidase pattern in this fraction could be obtained.

During the first 16 hr, peroxidase reaction to tissue cutting was significantly greater than to infection. In both inbreds, the main increase in the enzyme activity occurred in the cytoplasm and was accompanied by a relatively small increase in the ionically bound wall fraction. No significant differences were observed in the covalently bound fraction.

During the following 24 hr, the total peroxidase activity in the cut laminae of Tms and N cytoplasm inbreds showed an insignificant and small increase, respectively. In the infected laminae, the peroxidase activity increased greatly in the ionically bound fraction in the Tms inbred and in both soluble and ionically bound fractions in the N cytoplasm inbred. The increases in the covalently bound fractions were relatively small in both inbreds.

A significant, although smaller, increase in peroxidase activity, especially of the soluble fraction, was also found in the control laminae between 16 and 40 hr of incubation.

No new isoperoxidases were electrophoretically detected in either inbred in response to cutting, infection, or detachment alone and exposure to darkness at 28 C. Since the reaction of particular isoperoxidases to cutting or infection after 16 hr was similar to that observed after 40 hr and no significant differences was found between N and Tms cytoplasm inbreds in this respect, only changes in the isoperoxidase activity in the Tms cytoplasm inbred are presented in Table II.

No significant increases were found in the activity of anodic isoenzymes, either in the soluble or ionically bound fractions, except a small increase in A2, the dominant isoenzyme at time

0. However, this increase was similar in the treated as well as control tissues and seems to be due only to aging under experimental conditions.

Among the cathodic isoperoxidases only C1 did not react to cutting or infection; C3 and C4 contributed mainly to the increase in enzyme activity of the soluble fraction, whereas C2 and especially C5 contributed mainly to that of the ionically bound fraction. The diseased and mechanically injured tissues differed somewhat in the distribution of the two latter isoperoxidases. The protoplast-wall ratios of C2 as well as C5 were much lower in the diseased tissue than in the cut-injured one.

DISCUSSION

The two corn inbreds with identical nuclear genes, but differing in their resistance to the fungus, revealed the presence of 10 isoperoxidase bands in their young laminae; the electrophoretic mobility of the bands resembled that of the major isoperoxidases among the 24 found in various organs of 250 corn varieties (12). No differences were observed between the inbreds in the number or mobility of the detected isoperoxidases before or after inoculation with *H. maydis* race T. Thus, the possible involvement of cell peroxidase in the plant resistance to infection cannot be related to qualitative differences in its isoenzymic spectrum between the host plants. No data are available for the qualitative pattern of the peroxidase fraction covalently bound to the walls of corn leaf cells. In tobacco plants, its pattern was similar in three varieties, differing in their resistance to tobacco mosaic virus, and did not change in the infected tissue (4).

The two corn inbreds showed no qualitative differences before or after inoculation in the peroxidase activity, not only of the soluble fraction, but also of the wall-bound one. Moreover, no significant qualitative or quantitative differences were found between the isoperoxidase response of the two inbreds to infection and that to cut injury. The differences found after 16 hr were due to the delayed effect of fungal inoculation as compared with that of cut injury. Thus, this study indicates that changes in the cell isoperoxidase activity induced by infection may result from a nonspecific response to injury and supports other findings based on the reaction of soluble peroxidase (23, 27, 30).

Peroxidase is thought to protect the infected tissues in various ways. It may enhance oxidation of phenolic compounds to products toxic to the infecting agent or to the infected cells (2, 14, 24); in the latter case, necrotic foci prevent the spread of the infectious agent (11, 19, 20, 25, 26). It may increase the lignification of cell walls, rendering them impervious to degradation or penetration by the pathogen, or both (6, 8, 15).

This study clearly showed a sharp injury-induced increase of the peroxidase activity in the protoplast, followed by its increase in the cell wall, especially in the ionically bound fraction. No data are available for the free peroxidase in the corn cell walls. However, in infected and/or mechanically injured tobacco tissue, no significant increase in its activity was observed during the first 24 to 48 hr. The nonspecific reaction of cell peroxidase to injury may contribute to the plant protection against a parasite. Perhaps the recently reported differences in the reaction of soluble peroxidase in rice leaves to two different races of the blast fungus (29) may partially explain the differences in the host susceptibility to the two pathogen races. However, the level of peroxidase activity or the rate of its increase is not necessarily linked with the host resistance to a pathogen by a cause and effect relationship, in particular in the case of the two corn inbreds studied. The differences in the susceptibility of the N and Tms inbreds to *H. maydis* race T are correlated with differences between them in the susceptibility of their organelle membranes to host specific toxin (T-toxin) produced by the fungus (1, 16, 21). The T-toxin is considered to be the primary determinant of susceptibility (9, 17) and its action may precede the nonspecific reaction of peroxidase, especially if the wall peroxidase fraction plays a major role in the cell protection. One may also assume that the often observed relatively small increases of peroxidase activity in susceptible hosts may be a consequence of metabolic disorganization in the cell rather than the cause of a lower resistance.

We have clearly shown that corn leaf isoperoxidases are distinctive in their distribution in the cell as well as in their reaction to mechanical injury. Also, the early response of leaves to the infectious agent has been shown to be similar to the response to mechanical injury in respect to specific isoperoxidases and their distribution between the protoplast and cell walls. Thus, in the search for clues to the involvement of peroxidase(s) in cell or tissue resistance to pathogens, greater attention should be paid to these factors.

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