PEROXIDASE ACTIVITY AND ISOZYME PATTERN OF DIFFERENT CLONES/VARIETIES OF SUGARCANE *(SACCHARUM OFFICINARUM L.)* WITH VARYING RESISTANCE TO SMUT (CAUSED BY *USTILAGO SCITAMINEA* SYDOW)

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ABSTRACT

The association of peroxidase activity and isozyme pattern with smut resistance in sugarcane was studied. Co 440 (immune), HQ 409 (intermediate resistant), two immune, and two highly susceptible progenies of the two varieties, one resistant control, CAC 57-13, and one susceptible control Phil 56-226, comprise the eight clones investigated.

Peroxidase activity was found not to differ significantly among the clones. No significant correlation was observed between peroxidase activity of healthy tissue and healthy part of infected tissue and percent field infection.

Infected tissues showed alteration in their isozyme pattern as compared to that of healthy tissues; there was evident increase in peroxidase activity in some isozymes and a decrease in others. However, among the various clones, the isozyme pattern did not show distinct features to differentiate immune/resistant from susceptible clones.

The results indicate that peroxidase may not be involved in smut resistance of sugarcane and hence, may not be used as an index for such resistance.

INTRODUCTION

Smut, caused by *Ustilago scitaminea* Sydow, is one of the most destructive diseases of sugarcane in the Philippines and in other sugarcane growing countries in the world. At present, the use of resistant varieties is the only practical method of smut control.

Screening for smut resistance in sugarcane by the conventional field rating method becomes quite unreliable in the long run for there are bred

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varieties rated resistant which turn susceptible with time. The establishment of a reliable and stable criterion for resistance is therefore desirable.

A number of authors have tried to find a correlation between the peroxidase activity of healthy host plants and their resistance to diseases. Resistance in the field of varieties of *Solanum tuberosum* to late blight caused by *Phytophthora infestans* may be indicated, with some restrictions, by the peroxidase test (1). A decrease of peroxidase activity in potato leaves upon ringing the stem was observed to be accompanied by a decrease in resistance to *Phytophthora* disease (2). Siegel and Galston (3) have also found a correlation between high levels of peroxidase activity and resistance to disease infection. These observations indicate that the activity of phenol oxidizing enzymes such as peroxidase in healthy plants may be correlated with the degree of resistance.

Studies on young corn smut tumors showed high catalase activity which destroys hydrogen peroxide and also inhibits indole acetic acid (IAA) oxidase, thereby increasing the level of IAA in the plant (4). IAA oxidase may be any of the peroxidase isozymes which catalyze IAA oxidation (5). It has also been observed that sugarcane smut is characterized by an abnormal growth of the apical tissue (6) which possibly reflects high levels of IAA.

Based on the above observations peroxidase activity might be associated with sugarcane resistance to smut and hence, this relation might be used as a more rapid and more sensitive screening method for sugarcane clones/varieties resistant to smut.

This investigation involved determining peroxidase activity and isozyme patterns of healthy and infected tissues of eight sugarcane clones representing immune, intermediate resistant and susceptible specimens.

MATERIALS AND METHODS

Collection of Plant Materials. Two immune (nos. 8 and 12) and two very highly susceptible (nos. 7 and 13)progenies of the cross Co440 x HQ409 already standing in the field were selected on the basis of their rated reactions to smut (7). The parent varieties, Co 440 (no. 19, immune) and HQ 409 (no. 20, intermediate resistant), a resistant check, CAC 57-13 (no.21), and a susceptible check, Phil 56-226 (no. 22) were also used.

Threee sample collections were done with each collection corresponding to the field replication. For each clone, tillers of about the same age (approximately six months) were collected at random within the replication. Healthy tissues corresponding to the undifferentiated terminal portion (leaf) within the leaf whorl (spindle) were collected from the eight clones. In addition, infected spindles were also collected from intermediate and susceptible clones. The tissues were chilled in ice while in the field.

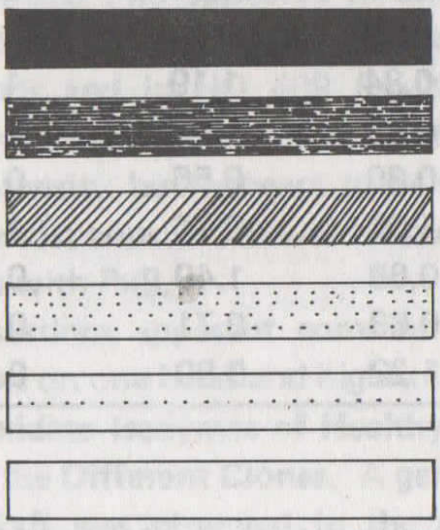
Preparation of Leaf Tissue Extracts. Healthy (H), healthy part of diseased (hd) and diseased (D) tissues were collected, washed thoroughly and weighed. Smut spores were carefully scraped off from diseased tissues. The samples in 0.1 M phosphate buffer pH 7.5 (1 g tissue: 3 ml buffer) were ground in a chilled mortar and centrifuged at 27,000 x g for 15 minutes at 4°C using a Sorvall RC5 refrigerated centrifuge.

Peroxidase Assay. Peroxidase activity was determined by recording the rate of decomposition of hydrogen peroxide by peroxidase with o-dianisidine as hydrogen donor (8). One unit of peroxidase activity is defined as that amount of enzyme decomposing 1 micromole of peroxide per minute at 25°C. Specific activity is defined as unit of activity per mg protein of crude extract. Protein was measured according to the method of Lowry *et al.* (9).

Determination of Isozyme Pattern by Disc Gel Electrophoresis. Electrophoresis of leaf extracts was done on 7.5% polyacrylamide gel by the modified method of Davis (10). Samples were mixed with glycerol on top of the stacking gel. Electrophoresis was carried out at 4°C and at constant current of 3mA/gel cylinder.

Peroxidase isozyme patterns were obtained by immersing the gels in 0.003% hydrogen peroxide-0.1% 0-dianisidine and fixed in 3% sodium pyrophosphate for 3 minutes and subsequently, washed and stored in distilled water (11, 12).

Band intensities are represented as follows:



very highly intense

highly intense

intense

less intense

least intense

hardly detectable

Numbering was done starting from the band nearest the cathode.

RESULTS

Peroxidase Activity. The peroxidase specific activity of H, hd, and D tissues and per cent smut infection of eight clones of sugarcane are shown in Table 1. The analyses of variance showed no significant difference in peroxidase activity of H, hd and D within clones or among clones. Thus, resistant and susceptible varieties did not differ in their peroxidase activity

before and after infection. In addition, peroxidase activities of H and hd were found not to be significantly correlated with field infection (r = 0.088; 0.31, respectively).

Table 1. Peroxidase specific activity of healthy (H), healthy part of diseased (hd), and diseased (D) tissue and percent smut infection of eight clones of sugarcane.

Clones No.	Rep[1]	Percent Smut Infection of Parent Clone	Peroxidase Specific Activity of Tillers [2]		
			H	hd	D
7	1	24.7	0.80	0.76	0.76
	2	41.0	0.80	0.78	0.55
8	1	0	0.79	-	-
	2	0	0.74	-	-
12	1	0	0.79	-	-
	2	0	0.87	-	-
13	1	30.0	0.52	0.68	0.63
	2	26.3	0.37	0.49	0.44
	3	34.4	0.95	0.92	1.06
19	1	0	0.80	-	-
	2	0	0.80	-	-
20	1	12.0	0.75	1.11	0.84
	2	0	0.76	-	-
	3	21.8	0.84	1.19	1.41
21	1	12.8	0.48	-	-
	2	0	0.80	0.56	0.51
	3	3.8	1.08	0.76	1.66
22	1	39.6	0.68	1.40	0.89
	2	29.7	0.63	0.71	0.77
	3	45.9	1.22	0.80	0.77

[1] Field replication

[2] H, healthy tissue; hd, healthy portion of diseased tissue and D, diseased (diseased part of infected tissue with spores scraped off).

Peroxidase Isozyme Patterns. Figure 1 shows the diagrammatic electrophoretic patterns of peroxidase isozymes of 8 healthy clones. No isozyme pattern was common to all clones although some clones showed closely related patterns, especially the progenies. They also resemble the female parent Co 440 (no. 19, immune).

There was difficulty in recognizing distinct bands due to diffused

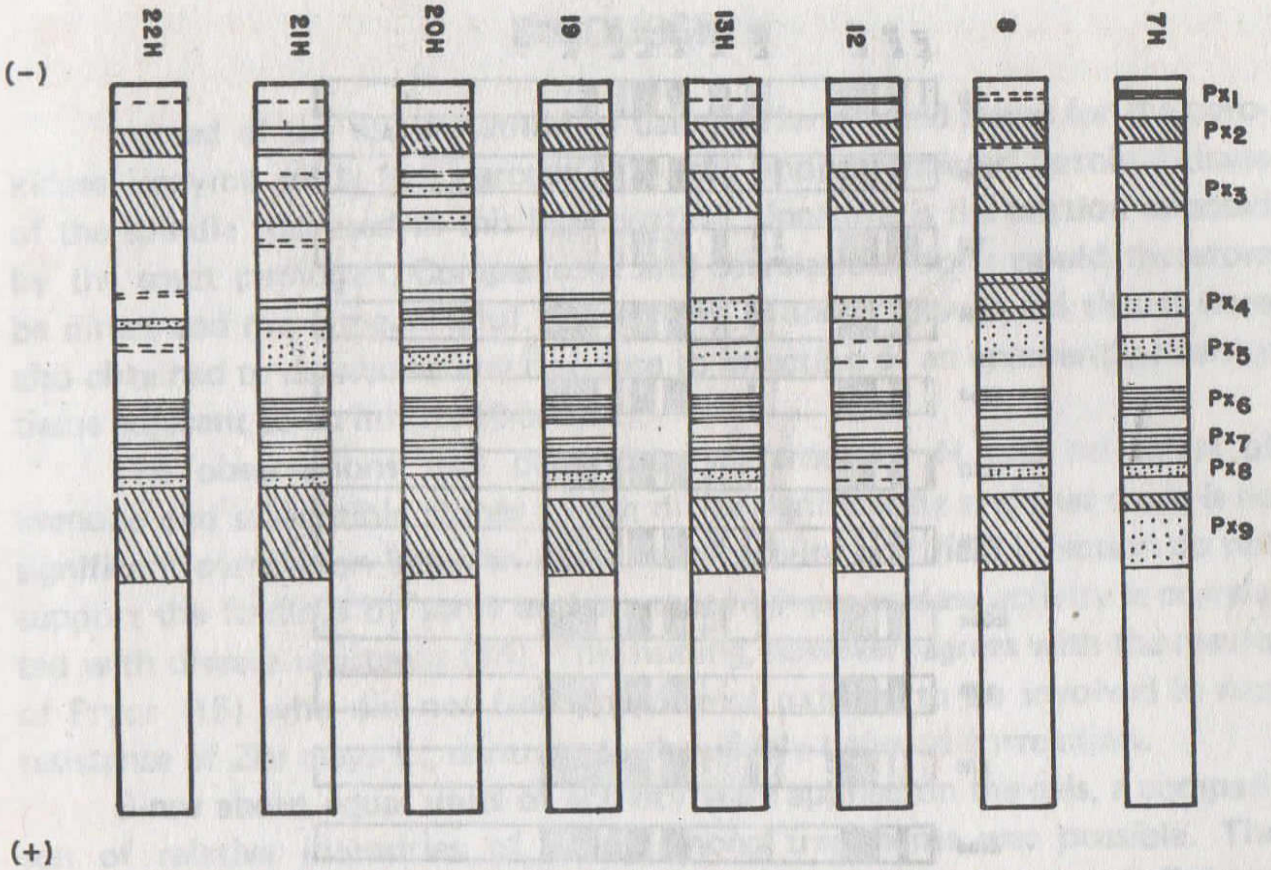


Figure 1. Diagrammatic sketch of peroxidase of the eight healthy sugarcane clones.

banding of peroxidase in the gel. However, 9-10 major bands were detected, with Px6 and Px7 prominent and consistent in width and intensity in all clones. Px2 and Px3 appeared in all clones as a single wide band except in CAC 57-13 (no. 21, resistant check) where Px2 was resolved into two narrow lighter bands and in HQ 409 (no.20, intermediate resistant male parent) where it was resolved into three bands. Px4 also appeared variable as to number and intensity but appears to be consistent with nos. 8, 19, 20, and 21. Px8 was almost constant in width except for 20, 21 and 22 in which Px8 was continuous with Px9.

No distinct apparent common feature was observed between all immune clones on one hand and highly susceptible clones on the other.

Peroxidase Isozymes of Healthy, Healthy Part of Diseased and Diseased Tissues of the Different Clones. A general loss of activity stain at band region Px4 and Px5 was observed in the gels of hd and D tissues of all clones (Figure 2). The appearance of new bands (region Px4-Px5) was also observed (13D, 21D, 22hd) while some bands increased in intensity (Px6 and Px7) and others decreased (region Px4-Px5). Px6 and Px7 of all infected clones (hd and D) showed greater intensity. This increased intensity may be interpreted to mean greater peroxidase activity of peroxidase isozymes with mobilities corresponding to these regions. Px2 intensity increased in all hd and D tissues but caution must be employed here since peroxidase isozyme band pattern of the pathogen also corresponds to this region so that a possible contamination during extraction could have been effected (Figure 3).

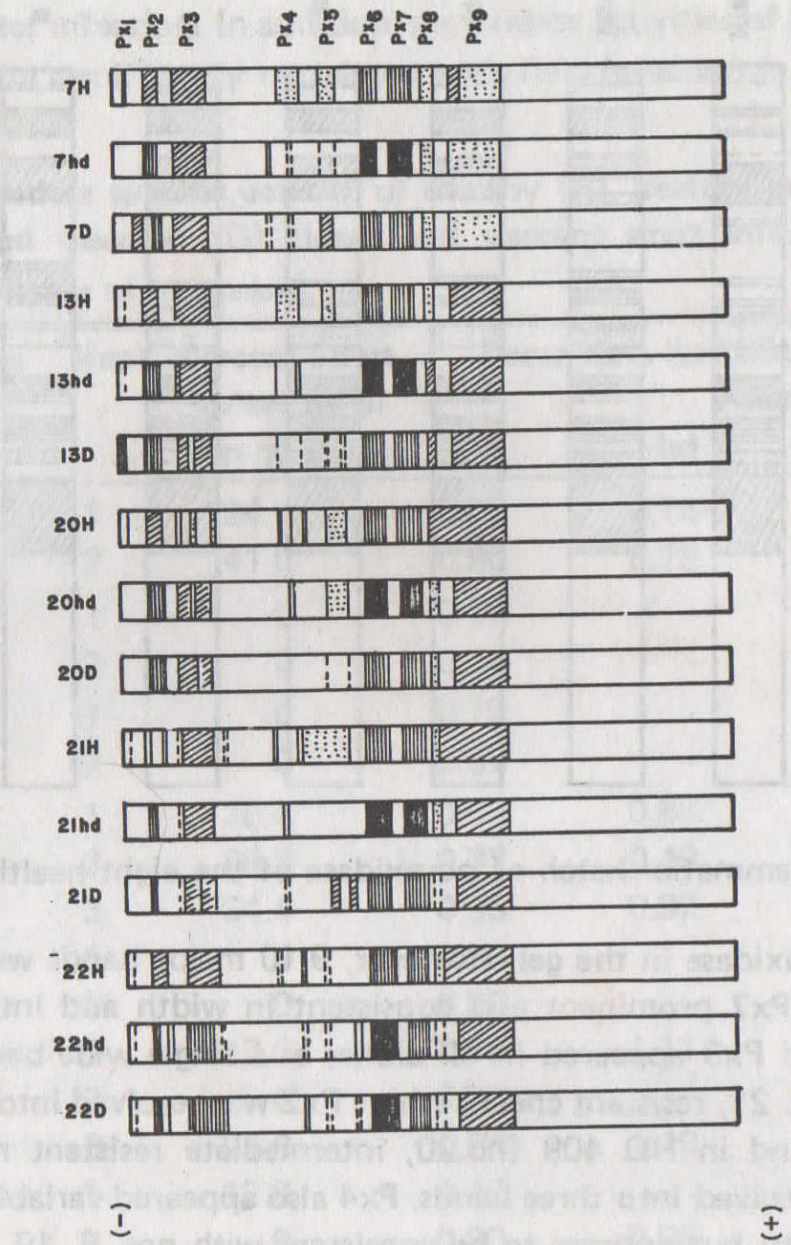


Figure 2. Diagrammatic sketch of peroxidase isozymes of healthy (H; healthy part of diseased (hd) and diseased (D) tissues of sugarcane.

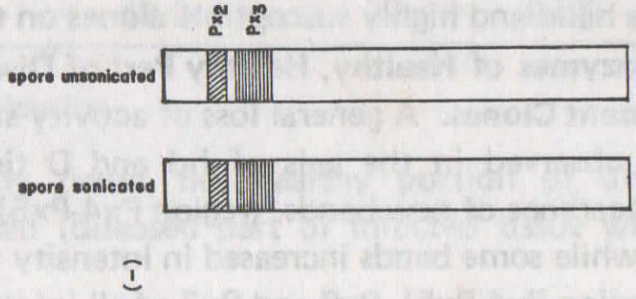


Figure 3. Diagrammatic sketch of peroxidase isozymes of the spores of *Ustilago scitaminea* Sydow.

Since no hd and D tissues could be obtained from the immune clones, the present comparison is limited only to clones predisposed to infection and hence no correlation between band patterns and resistance can be made.

DISCUSSION

Instead of the usual method of using differentiated leaves for the peroxidase isozyme study in sugarcane (13), the undifferentiated terminal tissue of the spindle was used in this investigation, since this is the portion infected by the smut pathogen. Comparisons and correlations done would therefore be direct and more meaningful. Aside from H and D tissues, hd tissues were also obtained to determine any response to infection of an apparently healthy tissue adjacent to an infected portion.

The observations that peroxidase activities of H and hd tillers of immune and susceptible clones do not differ significantly and that there is no significant correlation between peroxidase activity and field infection do not support the findings by some authors that high peroxidase activity is correlated with disease resistance (14). This finding, however, agrees with the results of Pryor (15) who did not find polyphenol oxidase to be involved in rust resistance of *Zea mays* L., contrary to the widely believed correlation.

Since about equal units of activity were applied on the gels, a comparison of relative intensities of bands among treatments was possible. The increased intensity, thus increased peroxidase activity, in some bands (hd and D) suggests that some genes which may be involved in the peroxidase synthesis for a particular physiological condition (e.g., disease) may have been activated or derepressed (16).

Shifts in the distribution of activity among the bands were apparent. Some alterations were, however, characterized by the appearance of new bands or the absence of previously present bands, instead of just change in intensity. This suggests that some peroxidase isozymes are operative only at a specific physiological condition. The disappearance of certain isozymes, however, may also be attributed to repression of some genes or alteration in the structure of the peroxidase isozyme molecule (16). Indole acetic acid (IAA) has been found to repress the appearance of particular peroxidase isozyme in pea stem (17). Possible interaction between IAA and peroxidase can not be ignored since symptoms of smut in sugarcane strongly suggest IAA mediation.

The many bands of sugarcane peroxidase isozymes detected electrophoretically as well as their diffused characteristic in gels with crude extracts from healthy tissues provided difficulty in their analysis. With the infected samples, the peroxidase activity and isozyme pattern might have been affected by peroxidase of the pathogen. To minimize any contamination, smut spores were very carefully scraped off the samples. Comparing the peroxidase activity of healthy and infected tissues shows that the peroxidase activity due to the pathogen was not significant (Table 1).

When subjected to disc gel electrophoresis, both extracts of sonicated and unsonicated spores gave peroxidase activity bands with mobilities similar

to those of Px2 and Px3. Notably, even extracts of immune/resistant plants had isozymes of Px2 and Px3 mobilities indicating the presence of peroxidase isozymes of similar electrophoretic properties in both plant and pathogen. These findings suggest that the greater intensity of bands at Px2 and Px3 of infected samples may be due to contamination with the pathogen. Other investigators also have noted that the pathogen *per se* may contribute new forms of its enzymes to the host plant (16). Intensification of these and other bands may, of course, be a plant response to the infection.

A possible application of the results of this type of study is in crop breeding and selection programmes. However, the results of this particular investigation indicate no correlation between the peroxidase activity of healthy sugarcane leaf tissue and field resistance to smut. Hence, peroxidase activity may not be used as an index (or as one of the indices) of resistance to smut caused by *Ustilago scitaminea* Sydow.

SUMMARY AND CONCLUSIONS

Peroxidase activity assay and disc gel electrophoresis were employed to determine the possible association of smut resistance in sugarcane with peroxidase activity and isozyme pattern. Eight clones were used with their corresponding smut rating: the two parents Co 440 and HQ 409 (immune and intermediate resistant, respectively), 2 immune and 2 very highly susceptible progenies of the cross, and resistant and susceptible variety checks. Peroxidase activities of the terminal undifferentiated tissue of the spindle of healthy clones were observed not to differ significantly. Similar results were obtained among H, hd and D tissues of clones predisposed to smut infection. Furthermore, no significant correlation was found between peroxidase activity of H and hd samples and percent field infection. Isozyme pattern of healthy clones did not show meaningful features useful in differentiating immune (resistant) from susceptible clones. Isozyme patterns of hd and D showed a characteristic disappearance of some activity bands, addition of some other bands, and changes in intensity in some regions indicating changes in peroxidase activity. The general pattern of response, however, as reflected by the new isozyme pattern of infected tissue, varied from clone to clone.

For further studies in the same line, it is suggested that the other enzymes, and their interactions with hormones especially IAA, be looked into. Up to this point it can only be concluded that peroxidase may not be involved in smut resistance and may not be used as an index for smut resistance in sugarcane.

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