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Peroxidase Isozyme Activity in Tomato Plants Infected by Tomato Yellow Leaf Curl Virus

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ABSTRACT

Plant peroxidases have been purported to play an important role in plant resistance to bacterial, fungal, and viral diseases. The positive correlations were found between the increase in peroxidase activity and disease resistance of plant tissue after infection. The objective of this study was to determine whether peroxidase activity and its isozyme pattern were altered by tomato yellow leaf curl virus (TYLCV) infection. The infection of TYLCV was obtained by feeding viruliferous white flies onto tomato (*Lycopersicon esculentum* Mill cv. Seeda) plant overnight. Ten to fourteen days after infection, peroxidase enzyme was extracted from leaf tissue and fractionated with 10 % polyacrylamide gel electrophoresis. The differences in peroxidase isozyme patterns and activity were found between healthy and TYLCV-infected plants. TYLCV-infected tomato plants contained fewer isozymes but with higher activity than healthy controls. This suggested that peroxidase isozymes could be an indicator of disease infection in tomato.

INTRODUCTION

The role of plant peroxidases in resistance to bacterial, fungal, and viral diseases has been extensively investigated (Birecka *et al.*, 1975a; Birecka *et al.*, 1975b; Jennings *et al.*, 1969 ; Lagrimini and Rothstein, 1987 ; Stahmann, 1967 ; Vance *et al.*, 1976). The levels of peroxidase and its isozyme patterns have been shown to be altered by stress, chemicals, and infections (Gasper *et al.*, 1982). Peroxidases are also induced by wounding and are presumably involved in the repair of damaged cell wall (Birecka and Milles, 1974).

The positive correlations were found between the increase in peroxidase activity or peroxidase formation and disease resistance of plant tissues after infection (Andreev and Shaw, 1965 ; Weber *et al.*, 1967 ; Weber and Stahmann, 1966). This suggests that peroxidase is involved in the development of plant disease resistance. Sweet potato roots infected by the fungus *Ceratocystis frimbriata* showed an increase in peroxidase activity and in resistance to black rot caused by this fungus (Clare *et al.*, 1966). When maize leaves were infected with *Helminthos-porium carbonum*, the resistant host exhibited higher peroxidase activity as compared to non-infected control (Jennings *et al.*, 1969). Vance and co-workers (1976) found a two-fold increase in peroxidase activity in Reed canarygrass leaf discs inoculated with *Helminthosporium avenae*. The peroxidase activity was histochemically localized in the wall near the site of attempted penetration. They proposed that the peroxidase may function in the biosynthesis of lignin at the site of attempted penetration. Inoculation of several kinds of fungus pathogenic to cotton into cotton bolls also stimulated peroxidase activity by 2 to 6 folds (Mellon and Lee, 1985). The injection of *Pseudomonas tabaci* into tobacco leaves increased peroxidase activity and induced the formation of new isozyme bands of this enzyme along with the increase in resistance to this pathogen. Injecting solutions of commercial peroxidase into the leaves also resulted in increased resistance to the disease (Lovrekovich, 1968). Bozarth and Ross (1964) found systemic-induced resistance to tobacco mosaic virus (TMV) in

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the hypersensitive Samsun NN tobacco plants related to peroxidase. The increase in peroxidase activity led to the production of toxic compounds which caused early killing of infected cells and early formation of a barrier to viral spread (Simons and Ross, 1970 ; Simons and Ross, 1971).

Despite the positive correlation between the increase in peroxidase activity and the increase in plant resistance to the pathogen during infection demonstrated by a number of studies, some researchers have shown no such correlation. The increased peroxidase activities in cucumber leaves infected with cucumber mosaic virus were found to be correlated with symptom severity and virus multiplication rather than resistance (Wood and Barbara, 1971; Barbara and Wood, 1972).

Tomato is an economically important food crop sensitive to diseases and plant pests. Viral diseases are one significant problem in tomato. There are various kinds of viral diseases causing the decrease in growth and production of tomato such as tomato aspermy virus (TAV), tomato black ring virus (TBRV), tomato spotted wilt virus (TSWV), cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), and tomato yellow leaf curl virus (TYLCV).

Tomato yellow leaf curl virus (TYLCV) is an important viral disease causing leaf curling, chlorosis, stunting, and decrease in production in tomato (*Sutabutra*, 1989). It can be transmitted by grafting and white flies (*Bemisia tabaci* Genn.). Very little information is available regarding this disease and the mechanisms of resistance induced by the infection.

Since peroxidase activity and its isozyme patterns are known to be altered by bacterial, fungal, and viral diseases, this study intended to determine whether peroxidase activity and its isozyme patterns were altered by TYLCV infection.

MATERIALS AND METHODS

Plant Materials and TYLCV Infection :

Tomato (*Lycopersicon esculentum* Mill cv. Seeda) plants were grown from seeds in the greenhouse with a 14-h photoperiod. After two weeks, they were separated into two groups; the healthy control group which was fed by non-viruliferous white flies (*Bemisia tabaci* Genn.), and the TYLCV-infected group. Infection of TYLCV was obtained by feeding 10 viruliferous white flies per tomato seedling overnight. Successful transmission was determined by symptom expression of tomato yellow leaf curl disease 10-14 days after infection.

Tomato cultures were derived from excised shoot apex or lateral buds of healthy and TYLCV-infected plants grown on agar medium containing Murashige-Skoog salts and 2 % sucrose (Lagrimini and Rothstein, 1987).

Extraction Procedure :

Every 0.2 g leaf tissue was ground in 1.4 ml of 0.1 M phosphate buffer, pH 7.5 in an eppendorf microfuge tube. The samples were then centrifuged at 10,000 rpm for 15 min and the supernatant was transferred to another microfuge tube and saved for peroxidase detection.

Electrophoresis :

Immediately after extraction, 40 ml samples were fractionated with 10% polyacrylamide gel electrophoresis submerged in 0.1 M tris-glycine buffer, pH 8.3. A current of 3 mA per sample was applied until the indicator band of bromophenol blue reached within 1 cm of the bottom of the gel.

Enzymatically active bands were identified by staining the gels for 15 to 20 min with a mixture of 20 ml of 2.9 mg/ml β-naphthol acetone + 8 ml 0.1 M tris-buffer, pH 4.0 + 1 ml 3% H_2O_2. Then the gels were fixed in 10% glycerine in 7% acetic acid to preserve the colored reaction product localizing the position of peroxidase isozymes.

RESULTS

Polyacrylamide gel electrophoresis showed differences in peroxidase isozyme patterns between healthy and TYLCV-infected plants (Fig. 1). The highest number of isozymes found in this study was 8 isozymes in diseased plants both from tissue culture (lanes 3 and 4) and greenhouse (lane 6). Healthy controls both from tissue culture (lanes 1 and 2) and from greenhouse (lane 5) contained 7 isozymes with the absence of band c. Plants infected by other TYLCV isolates (lanes 7, 8, 9, and 10) contained only 4 isozymes.

The variations in the activity of peroxidase in the extracts of healthy and diseased plants from tissue culture and greenhouse are also shown in Fig. 1. Diseased plants both from tissue culture (lanes 3 and 4) and from greenhouse (lane 6) had higher peroxi-

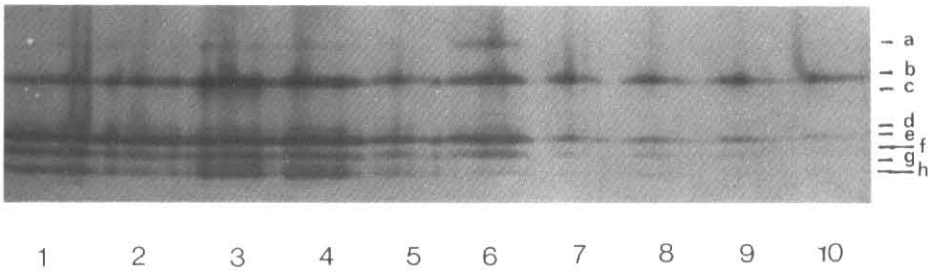

Figure 1 Peroxidase isozymes from leaf extracts of tomato electrophoresed on polyacrylamide gel and stained for peroxidase activity with β-naphthol. Lane 1 and 2, healthy plants from tissue culture; lane 3 and 4, diseased plants (severe strain) from tissue culture; lane 5, healthy plant from greenhouse; lane 6, diseased plant (severe strain) from greenhouse; lane 7, 8, 9, and 10 diseased plants (field isolate) from greenhouse.

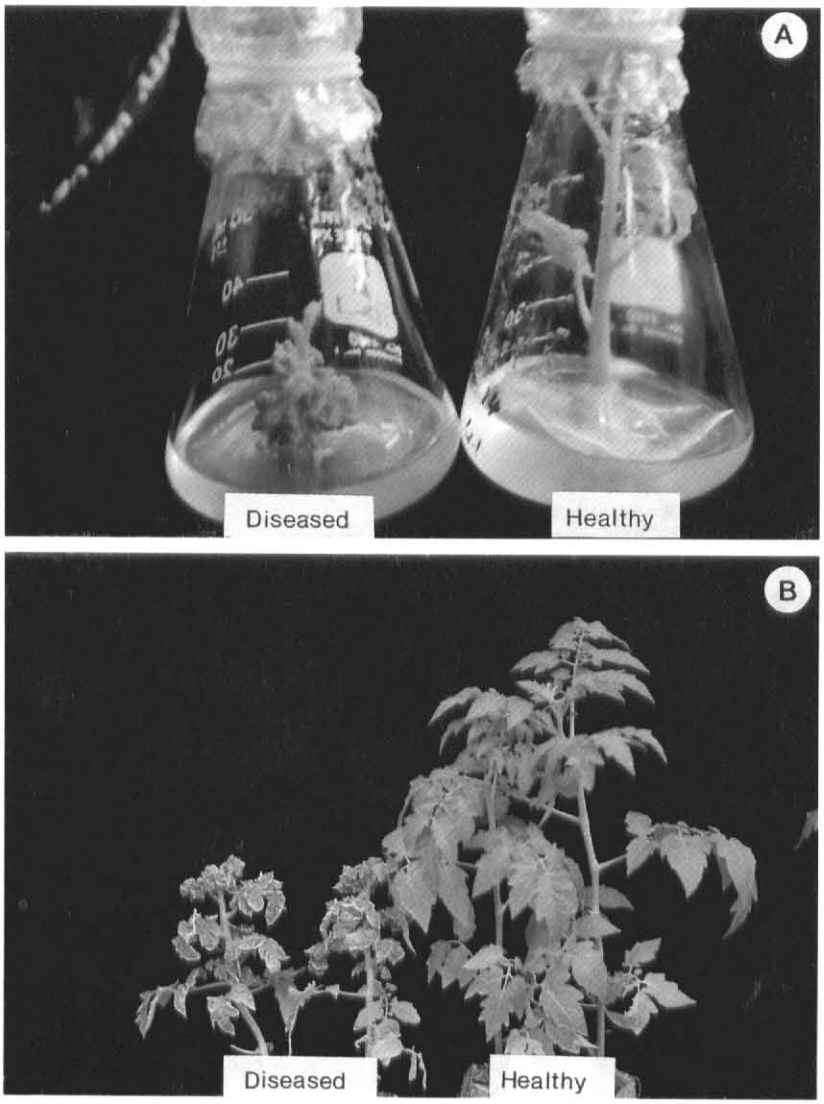

Figure 2 Healthy and diseased (severe strain) tomato plants from tissue culture (A) and from greenhouse (B).

dase activity than healthy controls (lane 1, 2, and 5) as demonstrated by having darker bands, especially bands a, d, and g. Peroxidase isozyme activity in plants infected by other TYLCV isolates (lanes 7, 8, 9, and 10) was obviously lower than those mentioned obove.

Plants infected by different isolates of TYLCV, both tissue-cultured and greenhouse-grown, showed symptoms of tomato yellow leaf curl disease (Fig. 2). The plants were small, compared to healthy controls. Leaves were curly and partly yellow. New leaves were small and curly.

DISCUSSION

The use of peroxidase isozymes as genetic and biochemical tools in the study of plant response to diseases is well established (Birecka *et al.*, 1975 a,b; Clare *et al.*,1966; Gasper *et al.*, 1982; Mellon and Lee, 1985). Tomato yellow leaf curl virus causes the decrease in growth and production of tomato. This problem is of great interest in Thailand. To be able to develop tomato plants resistant to TYLCV, significant indicator for resistance must be studied. Since it was suggested that peroxidase isozyme activity increases as plant resistance to disease increases (Clare *et al.*, 1966; Weber *et al.*, 1967), This study intended to determine whether pattern and activity of peroxidase isozymes were altered with TYLCV infection.

This study indicated that pattern and activity of peroxidase isozymes were altered with TYLCV infection (Fig. 1). Both number and activity of isozymes were higher in diseased than in healthy control plants. This corresponded with other researchers who found increases in peroxidase isozyme activity with infection of various kinds of diseases.

Isozyme activity of both healthy (lane 1, 2) and diseased (severe strain ; lane 3,4) plants from tissue culture was higher than that of the plants from greenhouse (lane 5 and 6). This may be due to the fact that there was sufficient exogenous nutrient and hormone supply in tissue culture system so that the plants could express defense mechanism by increasing isozyme activity. In contrast, the plants from greenhouse could be under other kinds of stress such as nutrient deficiency beside disease infection. Therefore, TYLCV infection accompanied with other stresses could cause severe damage to the plants and consequently caused depletion or weakening of host defense mechanism.

It is obvious that pattern and activity of peroxidase isozymes changed with TYLCV infection. This suggested that peroxidase isozymes could be indicator of disease infection in tomato. Whether or not changes in pattern and activity of peroxidase isozymes correspond with changes in plant resistance to TYLCV has to be investigated further. For example, early event response of isozyme pattern and activity to TYLCV infection must be observed. The information from this study would then be useful for improvement of tomato for resistance to TYLCV.

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