Acta Phytopathologica Academiae Scientiarum Hungaricae, Vol. 12 (3–4), pp. 151–156 (1977)

Heat-induced Local Lesions with High Peroxidase Activity in a Systemic Host of TMV

By

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Local necroses were induced in leaves of virus-infected Xanthi tobacco, a systemic host for TMV, by hot-water-treatment (50°C for 40 sec) 2–5 days after inoculation. Peroxidase activity was augmented during necrogenesis. The increased perioxidase isozymes were present also in the control samples, however, enzyme activities were very high in the necrotic parts of the leaf. The heat-induced necroses and the parallelly augmented peroxidase could not stop systematization of the virus. Neither tissue necrosis, nor increased peroxidase activity are responsible for virus localization, i.e. for host resistance.

The role of hypersensitive necrosis in plant disease resistance accompanied by augmented peroxidase and polyphenoloxidase activities has been a disputed question in the past decade (cf. GOODMAN *et al.*, 1967). The primary role of the hypersensitive response was questioned in the case of fungus diseases (BROWN *et al.*, 1966; SEEVERS *et al.*, 1972; KIRÁLY *et al.*, 1972; ÉRSEK *et al.*, 1973; BARNA *et al.*, 1974; MAYAMA *et al.*, 1975; TANI *et al.*, 1975) as well as in diseases caused by bacteria (KIRÁLY *et al.*, 1977). In resistance to viral infections an additional importance has been attributed to peroxidase enzyme because of its supposed role in the systemic acquired resistance (SIMONS and ROSS, 1970). However, the latest results reported on the absence of a relationship between peroxidase activity and plant viral resistance (BIRECKA *et al.*, 1975; WESTSTEIJN, 1976). Recently FOSTER and ROSS (1975a, b) called attention to the possibility of induction of tissue necrosis in the systemically infected Turkish tobacco leaves. In this case the host plant remained systemic to TMV in spite of the appearance of necrotic lesions.

The aim of our work was to measure the activity of peroxidases in the development of this "artificial necrosis" and have information on the eventual role of peroxidase in virus localization, e.g. in resistance to the virus.

Materials and Methods

Tobacco plants (*Nicotiana tabacum* L. cv. Xanthi) were grown under normal greenhouse conditions and used for virus inoculation in the 6–8 leaf stage. The U_1 strain of tobacco mosaic virus (TMV) was cultured in *Nicotiana tabacum*

L. cv. Samsun. Tobacco leaves showing the typical disease symptoms of TMV were ground (1 g leaf per 10 ml 0.01 *M* phosphate buffer) with a pestle and mortar and the homogenate was used to inoculate the plants. No abrasive was added to the inoculum. All of the half leaves of tobacco plants were inoculated. Forty-eight hours after inoculation the lower half of leaves was immersed into hot water (50°C for 40 seconds) (FOSTER and ROSS, 1975a). After treatment the plants were put into the greenhouse. All experiments were repeated ten times using 6–8 plants.

Leaf material for the assay of peroxidase activity was taken from four parts of each leaf; namely (a) absolute control, (b) heat treated, (c) systemically infected, (d) systemically infected and heat treated parts (cf. Fig. 1). Using a cork-borer, samples were taken, 3 g each, and were homogenized with quartz sand in ice-cooled mortar and pestle in 12 ml 0.2 *M* acetate buffer pH 5.6. The slurry was centrifuged at 6000 *g* for 20 min at 0°C. The supernatant was used for the assay of peroxidase activity. Peroxidase activity was measured spectrophotometrically in a Unicam SP 800 spectrophotometer. Pyrogallol was used as a substrate. The enzyme extracts occurred in the optimal concentration (0.2 ml) in the final volume of 3 ml reaction mixture. Measurement was made at 430 nm, and enzyme activities were expressed as the per cent increase in absorption between 15 and 45 seconds after the enzyme extract was added. Data were related to the untreated and uninoculated control.

Peroxidase activity was expressed both on a fresh weight basis and on a protein basis. For determining the protein content the method of LOWRY *et al.* (1951) was used. An aliquot of the extracts was used for polyacrylamide gel electrophoresis. Amounts of 100–200 μg protein were layered on gel. The gel electrophoresis of Davis (1964) was followed at pH 8.3. Samples were run in 0.01 *M* tris-glicin buffer (30 min 2 mA and 120 min 5 mA per tube). The gels were soaked in benzidine saturated 0.2 *M* acetate buffer, pH 5.6 and to the reaction mixture H_2O_2 was added (GÁBORJÁNYI *et al.*, 1973).

Peroxidase activities were measured immediately, 1, 2 and 3 days after the heat treatment, during the heat-induced local lesion development.

Results and Discussion

Using the technique of FOSTER and ROSS (1975a) we were able to induce necroses by hot water treatment in TMV-infected but symptomless half leaves of Xanthi, a compatible host for TMV (Fig. 1). As is seen, the local necroses appeared only on the TMV-inoculated and heat-treated parts of the half leaves. The treatment consisted of immersing the upper part of leaves into hot water of 50°C for 40 seconds, 2–5 days after inoculation. Necrotic lesions appeared 24–48 hours after the hot water treatment. During this time period the activity of peroxidase enzyme was measured and compared to the uninoculated but heat-treated control (Fig. 2). Neither the systemic infection, nor the hot water treat-

ment induced significant changes in activity of peroxidase during that time period. On the other hand, peroxidase activity was gradually augmented during necrogenesis. Increase in peroxidase activity in local lesion hosts as a result of virus infection is well known from the literature (FARKAS *et al*,. 1960; LOEBENSTEIN and LINSEY, 1961; ROSS, 1961a, b; FARKAS and STAHMANN, 1966; SOLYMOSY *et al.*, 1967; NOVACKY and HAMPTON, 1968; CHANT and BATES, 1970; SIMONS and ROSS,

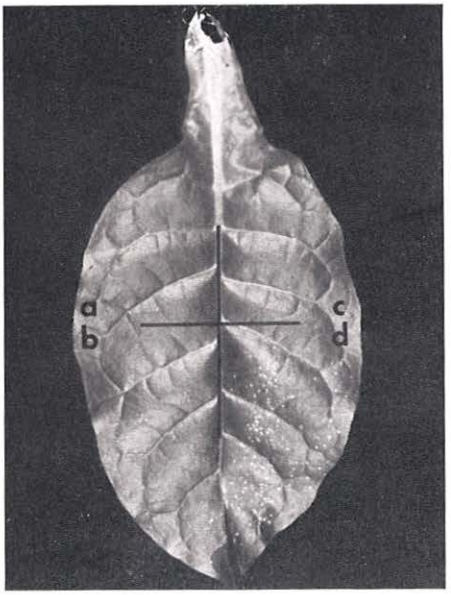

Fig. 1. Induction of necrotic lesions in "systemic Xanthi" tobacco leaf inoculated with TMV by heating the leaves in hot water at 50°C for 40 sec, two days after inoculation; (a) Uninoculated, untreated with heat; (b) uninoculated, heat-treated; (c) inoculated with TMV, untreated with heat; (d) inoculated with TMV and treated with hot water

1970; VAN LOON and GEELEN, 1971; WOOD and BARBARA, 1971; GÁBORJÁNYI *et al.*, 1973; BIRECKA *et al.*, 1975; VEGETTI *et al.*, 1975; VAN LOON, 1976; WESTSTEIJN, 1976).

Peroxidase samples from the heat-induced "artificial necroses" were electrophoretized. It was found that the so-called new peroxidase isozymes increased during the necrogenesis (Fig. 3). However, if we used enough quantities of samples, as was recommended by BIRECKA *et al.* (1975), it was clearly shown that the new peroxidases are present in all of the samples including the control ones. The activity of these isozymes was very high in the necrotic parts of leaves as compared to the controls. This means that the development of "artificial necroses" is also related to augmented peroxidase activity. This type of necroses could not stop virus movement. It was found that systemic distribution of the virus occurred in

the leaves, in spite of the development of local lesions. By this way we successfully repeated the findings of FOSTER and ROSS (1975a, b).

As a conclusion of these experiments we suggest that both peroxidase activity and the hypersensitive necroses (local lesion) are consequences, and not causes, of host resistance to viral infection. Similar conclusions have been made recently

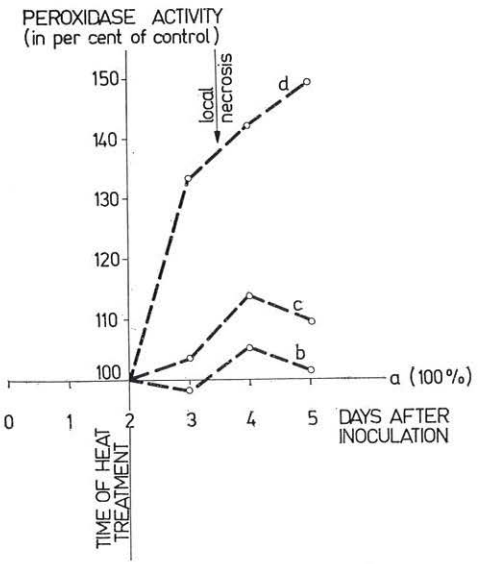

Fig. 2. Activity of peroxidase enzyme in per cent of uninoculated and non-heat-treated control leaf part (a). (b): Uninoculated, treated in hot water at 50°C for 40 sec two days after inoculation with TMV. (c): Virus-inoculated, untreated with hot water. (d): TMV-inoculated and hot water-treated

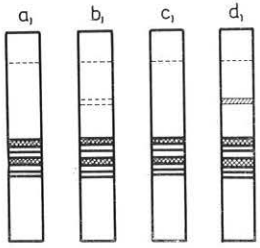

Fig. 3. Diagramatic representation of the distribution of the major peroxidase isozyme components following electrophoresis of soluble protein extracts from (a): uninoculated, untreated with hot water; (b): uninoculated, treated with hot water at 50°C for 40 sec two days after inoculation with TMV; (c): virus-inoculated, untreated with hot water; (d): inoculated with TMV and treated with hot water

in relation to host resistance to fungal infections (BROWN *et al.*, 1966; SEEVERS *et al.*, 1972; KIRÁLY *et al.*, 1972; ÉRSEK *et al.*, 1973; BARNA *et al.*, 1974; MAYAMA *et al.*, 1975; TANI *et al.*, 1975).

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