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Growth Inhibition of Virus-infected Plants: Alterations of Peroxidase Enzymes in Compatible and Incompatible Host-Parasite Relations

By

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The rate of growth inhibition of inoculated tobacco cultivars, *Nicotiana tabacum* L. cv. Samsun and cv. Xanthi-*nc* was measured one month after systemic infection with tobacco mosaic virus (TMV), U_1 strain and cucumber mosaic virus (CMV) white strain, respectively. A positive correlation was determined between the virus-induced host stunting and the enhanced activity of peroxidase enzymes of the host stem. The systemic infection did not induce synthesis of new peroxidase isozymes. The growth inhibition of the TMV-infected hypersensitive cultivar (Xanthi-nc) was not remarkable. Similarly, no significant change was experienced in this case in the peroxidase activities, except the soluble fraction of the inoculated leaves. New peroxidase isozymes appeared in the soluble and cell-wall bound fraction of the inoculated leaves, but there was no change in the isozyme spectra of the stem fractions. Permanent effect of the local virus infection on the peroxidase activity was detected only in the soluble fraction of the young, non infected leaves, which may be in relation to the systemic acquired resistance. No change of activities was registered in the peroxidase fractions of the stem, according to the nearly normal growth of the host. The dwarfing effect of systemic virus infection was reversed by treatment with gibberellic acid. Since gibberellin treatment may reduce indolaecetic acid oxidase activity in the plant cell-wall fraction one can suppose that the virus-induced dwarfing effect could be reversed by this way.

The pathogen increases without significant limitation in virus infected susceptible host plants inducing a systemic type of infection (compatibility). The systemic infection may be symptomless or visible symptoms may be found such as mosaic, chlorosis, leaf and flower abnormalities. One of the most characteristic symptoms is the inhibition of growth of diseased plants. This growth inhibition can be expressed by reduction of leaf size and the shortening of internodia. In most cases dwarfing induced by virus infection is the cause of yield loss having significant economic importance (Cf. HORVÁTH, 1971). In spite of this the metabolism of growth inhibition induced by virus infection has not been clarified from a pathophysiological point of view.

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Resistant host plant reacts to the infection of pathogen by a hypersensitive reaction and localizes it in the site of infection (incompatibility). In this host-parasite relationship (resistant plant) growth inhibition can not be expected or if so to a limited extent.

The explanation of the significant difference in the symptoms of the two host-parasite relationships may be connected to different metabolic changes in the host plant. The different metabolism of local and systemic virus infections has been reported (FARKAS, KIRÁLY and SOLYMOSY, 1960; FARKAS and SOLYMOSY, 1965; GOODMAN, KIRÁLY and ZAITLIN, 1967). The intention was primarily centered on the cause of the resistance. Particularly, the increased activity of peroxidase was involved in the formation in active zones surrounding of local lesions (ROSS, 1961a and b).

The indoleacetic acid oxidase enzyme – indirectly regulating growth – was found to be identical with peroxidase (RAY, 1958). Determining its localization in the cells (HACKETT and RAGLAND, 1962; RIDGE and OSBORNE, 1971; SÁGI, 1970, 1971) provided the opportunity for the investigation of this enzyme not only in the soluble, but in the cell-wall bound fraction too.

The peroxidase activity of virus infected plants has been measured in many cases. However, former investigations did not extend to the separation of certain peroxidase fractions, on the other hand the obtained results were not related to the abnormal growth due to virus infection.

In the present study the activity of peroxidase was examined in the leaf and stem tissues. Our results indicated, that after the systemic virus infection the activity of peroxidase enzyme increases permanently both in stem and leaf in all of the fractions, without the formation of new isozymes. On the contrary in resistant host plants, in the course of the hypersensitive reaction, marked activity can be detected both in the soluble and the cell-wall bound fraction of infected leaves. This, however, is accompanied by the appearance of new isozymes. New isozymes do not occur in the bound and soluble fractions of the stem. The rise of peroxidase activity in stems is only temporary.

Material and Method

Host-parasite relations. For investigating of induced growth inhibition by virus infection tobacco (*Nicotiana tabacum* L. cv. Samsun and cv. Xanthi-*nc*) seems to be a suitable material. Tobacco plants, grown under normal greenhouse conditions, were used for virus inoculation in the 6–8 leaf stages.

Tobacco mosaic virus (TMV, U_1 strain) and cucumber mosaic virus (CMV, white strain) were used for inoculations. One month after virus infection remarkable growth inhibition was detectable in the compatible host-parasite relation. A hypersensitive tobacco (Xanthi-*nc*) was applied as local host plant for TMV infection. Estimation of growth inhibition of diseased plants and investigation on

the activity of peroxidase enzyme were carried out on systemically infected plants one month after inoculation.

In the case of local infections peroxidase activity was measured 5–6 days after inoculation i.e. at the very time of symptom development. For investigating the permanent effect of local virus infection on peroxidase of this enzyme was measured one month after inoculation in the non-infected leaves and stems. The measurements were carried out in each season.

Peroxidase. For examining of peroxidase activities the samples were taken from upper third of plants. Ten grams of fresh weight of leaf and stem samples were ground in equal volume of cold distilled water in mortar and then were homogenized in a Waring blendor. After centrifugation of aqueous extract (1500 rev/min, for 10 min) the supernatant was separated. The sediment was suspended again in distilled water, ground in a mortar and centrifuged again. The supernatant fractions were collected in 8 replications. The 8–10. fractions having no more peroxidase activity were discarded. The collected supernatants were centrifuged in a preparative ultracentrifuge (Janetzki VAC 60 type, 105.000 g, for 1 h) and the supernatant was used for measuring the soluble peroxidase activity.

The cell-wall bound peroxidase activity was measured in the residue of distilled water fractionation, with a phosphate buffer (0.15 *M*, pH 7.2) containing 0.3 *M* NaCl. The activities were measured according to the method of BELOSERSKI and PROSKURJAKOV (1956). 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. The dialized enzyme extracts were concentrated in 50% polyethylenglycol (Carbowax 20.000). Amounts equivalent to 100–200 μg protein were taken on gel (Cyanogum 41.5%) and were run in 0.1 *M* TRIS-EDTA buffer (30 min 2.5 mA and 90 min 5 mA per tube). The gels were soaked in 0.2 *M* Na-acetate buffer (pH 5) and the peroxidase activities were determined by incubation in solutions of benzidine and hydrogen peroxide.

Results and Discussion

Growth inhibition induced by virus infection

Systemic mosaic symptoms of TMV and CMV appeared 12–15 days after inoculation. Growth inhibition could be observed from the second week following inoculation. The number of leaves developed on the infected plants was similar as on the control, however, the internodia became shorter. The growth inhibition induced by virus infections are summarized in Table 1.

The resistant host plants react to virus infection by developing local necrosis (incompatibility). Local necroses can be seen from the second day following infection. At the high inoculum concentration used by us 50% of the leaf surface

Table 1

Growth and leaf weight of healthy and virus infected plants*

Host-parasite relation	Stem length (cm)	Leaf weight (g)
TMV–Samsun (compatibility)	8.6	61.5
Control Samsun	19.0	89.0
CMV–Xanthi (compatibility)	9.5	50.0
Control Xanthi	16.3	101.5
TMV–Xanthi (incompatibility)	16.5	88.5
Control Xanthi	17.9	99.6

* Measures one month after inoculation
TMV = tobacco mosaic virus; CMV = cucumber mosaic virus

necrotized. One can see in Table 1 that the growth of resistant Xanthi plants were not significantly influenced by TMV-infection.

Changes in peroxidase activity

Investigations so far have shown that the oxidative metabolism of systemically infected leaves slightly increases with the appearance of symptoms. In certain cases it is in correlation with virus multiplication (LOEBENSTEIN and LINSEY, 1961; NOVACKY and HAMPTON, 1967; WOOD and BARBARA, 1971; STAHMANN and DEMOREST, 1972). According to our measurements (Table 2) peroxidase activity

Table 2

Peroxidase activities in susceptible host-parasite relation

Plant part	Peroxidase fraction	Peroxidase activity*	
		TMV–Samsun	CMV–Xanthi
Leaf	soluble	1.30	0.99
	bound	1.49	1.60
Stem	soluble	2.69	1.80
	bound	2.07	1.32

* Specific activity rates presented per 10 μg protein
TMV = tobacco mosaic virus; CMV = cucumber mosaic virus

showed a slight rise in the leaves, both in the cytoplasmic and the cell-wall fractions. Only the activities in the cytoplasmic fraction make a comparison with date of literature possible. The so-called "soluble fraction" does not contain ribosomal or mitochondrial peroxidase. In this way our results are in accordance with other

data, although in our experiments the values are generally lower. This is especially the case in Xanthi-TMV relationship.

The peroxidase enzyme activities measured in the stem, similarly showed an increasing tendency, which proves the permanent efficiency of a general stress effect connected with infection. Activities measured in soluble and bound fraction of the infected plants rise by about twofold as compared to the control. The increased peroxidase activity measured in the stem could have a remarkable role in the oxidation on indoleacetic acid responsible for cell elongation.

In the case of resistant plants the necrogenic reaction connected with virus infection activates a series of enzymes in infected leaves both at the site of necroses and in the zones surrounding the lesions (SOLYMOSY and FARKAS, 1963). The peroxidase activities show a maximum at the very time of symptom development (VAN LOON and GELEEN, 1971).

Peroxidase activities established 5–7 days after inoculation in the inoculated leaves are shown in Table 3. The data obtained at time of symptom appearance

Table 3

Peroxidase activities in incompatible host–parasite relation

Plant part	Peroxidase fraction	Peroxidase activity*	
		TMV-Xanthi**	TMV-Xanthi***
Leaf	soluble	3.60	1.29
	bound	1.29	0.98
Stem	soluble	0.95	0.72
	bound	1.44	1.02

* Specific activity rates presented per 10 μg protein
** Measurement 6 days after inoculation
*** Measurement 30 days after inoculation in the newly developed parts

demonstrate deviations according to the enzyme source. The marked increase in the activity of the soluble fraction of inoculated leaves is well-known from the literature. Much more important is the fact that the peroxidase activity of other leaf or stem fractions shows only a slight change.

Our data are in accordance with symptom observations. After inoculation a stress effect is going on in the resistant plants and if the inoculation is accompanied by necrosis the host may lose one or more leaves. Thus, disturbances in growth are only temporary in the host, therefore plants can grow normally. According to SIMONS and ROSS (1970, 1971) peroxidase activity in the upper non-infected leaves of locally infected host plant remains at a high level three weeks following inoculation. In the newly developed healthy leaves the peroxidase activity is in

correlation with the systemic acquired resistance. As regards enzymes other than peroxidase such lasting effect could not be observed.

This results are apparently in contradiction with the quite normal development of infected plants. If the peroxidase activities are measured in the cell-wall bound and soluble fractions both in the leaves and the stems, this contradiction disappears. One month after infection peroxidase activities are smaller than the values obtained during the lesion development. The activities in bound fraction or the stem fractions are similar to the control. An increase in the enzyme activity occurs only the enzyme fraction having no importance from the point of view of stem elongation.

Peroxidase isozymes

Not only the activities of peroxidase enzymes show changes as a result of virus infection, but the number of components are also altered. According to the data so far, the enzyme composition in systemic host-parasite relations does not change, but in the case of local lesion hosts new isoenzymes appear after infection (LOEBENSTEIN and LINSEY, 1961; FARKAS and STAHMANN, 1966; VAN LOON and GEELEN, 1971; STAHMANN and DEMOREST, 1972). In contrast to these observations NOVACKY and HAMPTON (1967; 1968) and CHANT and BATES (1970) found that in the local lesion hosts the "new" peroxidase isoenzymes are only the already existing isoenzymes activated during the process of senescence. FARKAS and STAHMANN (1966) and STAHMANN and DEMOREST (1972) also found new isoenzymes related to senescence, but among these was one new isozyme which occurred only in virus infected plants. The development of the new isozyme can be prevented by protein inhibitors.

It must be stressed that the distribution of peroxidase isozymes in virus-infected plants was investigated in the above-mentioned cases according to a quite different experimental design. So there is no possibility to compare the data of the literature with our results except one field e.g. the cytoplasmic fraction (SOLYMOSY *et al.*, 1967).

As was expected, no new isoenzymes appear in the cytoplasmic fraction of the leaf of systemically infected tobacco (Fig. 1). Only the different discolouration of certain isozymes refers to the enhanced peroxidase activity of infected plants. Similar results were obtained in the other fractions, too. Results of figures are only representative ones. Measurements were repeated several times, during the four seasons, but in the systemic infection of TMV and CMV we have never found new isoenzymes, although there were some differences in the number of isoenzymes. MCCOWN *et al.* (1969) also reported on similar alterations in the isoenzyme spectra in relation to the seasons. Certain isozymes of systemically infected plants showed markedly high activity which is in accordance with the spectrophotometrical investigations.

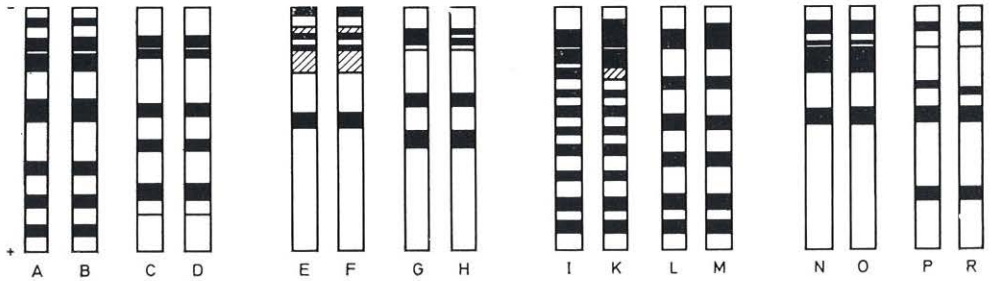

Fig. 1. Peroxidase isozyme spectrum in compatible host-parasite relation, in the leaf and stem fractions of systemically infected tobaccos
Leaf soluble: A TMV — Samsun; B C — Samsun; C CMV — Xanthi; D C — Xanthi.
Leaf bound: E TMV — Samsun; F C — Samsun; G CMV — Xanthi; H C — Xanthi.
Stem soluble: I TMV — Samsun; K C — Samsun; L CMV — Xanthi; M C —Xanthi.
Stem bound: N TMV — Samsun; O C — Samsun; P CMV — Xanthi; R C — Xanthi.
TMV = tobacco mosaic virus; CMV = cucumber mosaic virus; C = control

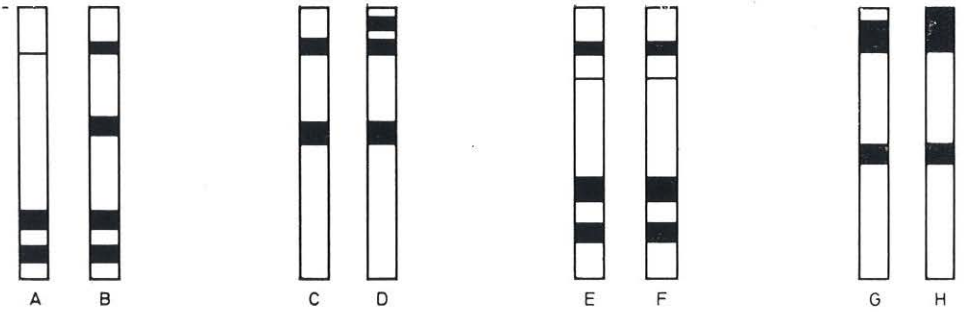

Fig. 2. Peroxidase isoenzymes in incompatible host-parasite relation, in the stem and leaf fraction of local infected tobaccos
Leaf soluble: A C — Xanthi; B TMV — Xanthi; Leaf bound: C C — Xanthi; D TMV — Xanthi. Stem soluble: E C—Xanthi; F TMV — Xanthi. Stem bound: G C — Xanthi; H TMV — Xanthi

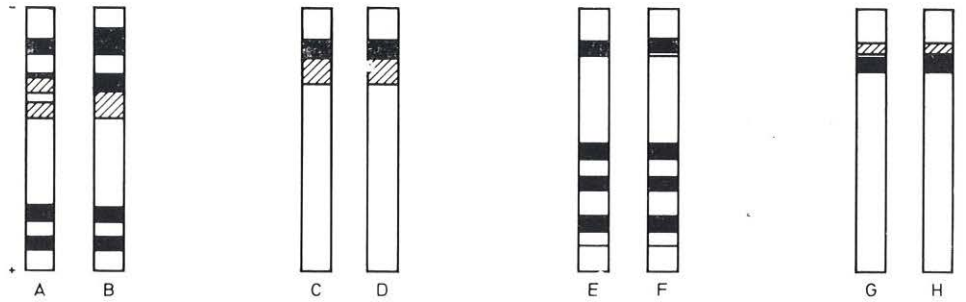

Fig. 3 Peroxidase isoenzymes in incompatible host-parasite relation 5 and 30 days after inoculation
Leaf soluble: A Inoculated leaf; B Newly developed leaf. Leaf bound: C Inoculated leaf; D Newly developed leaf. Stem soluble: E Five days after inoculation; F Thirty days after inoculation. Stem bound: G Five days after inoculation; H Thirty days after inoculation

In the resistant plants new isoenzymes appear as a consequence of local virus infection, not only in the cytoplasmic but also in the cell-wall bound fraction, indicating significant change in the enzyme system of the cell-wall fraction. Two new isozymes appear in the soluble and one in the cell-wall bound fraction (Fig. 2).

Especially important is the fact that the new isozymes induced by TMV are partly cationic peroxidases. We should like to note that one cationic isozyme of horse-radish peroxidase is more active in ethylene production than the anionic ones (YANG, 1968). The rise of peroxidase activity in hypersensitive tobaccos may be related to the enhanced ethylene production of infected plants (GÁBORJÁNYI, BALÁZS and KIRÁLY, 1971; NAKAGAKI and HIRAI, 1971).

We have not found new peroxidase isozymes in stem fractions, which mean that in this case the virus infection has only local effect on peroxidases. After a one-month-infection isoenzyme changes were not observed in the stem of resistant host plant compared with the control, whereas isozymes of soluble and bound peroxidase showed a higher activity than the control (Fig. 3). This higher activity is connected with the increased activity of peroxidase measured spectrophotometrically. Permanent effect of virus infection, therefore, is detectable only in the newly developed leaves.

Reversion of induced dwarfing

The dwarfing effect induced by systemic virus infection may be due to peroxidase enzymes (primarily to the indoleacetic acid peroxidase). One can suppose that by treatment with hormones, the stunting caused by the virus can be reversed. For this purpose we treated tobacco plants systemically infected with cucumber mosaic virus with indole-3-acetic acid, 3-indolyl-acetamide, 5-hydroxy-indole-3-yl-acetic acid, gibberellic acid (GA_3), benzyladenine four times, weekly. The inhibition of growth caused by virus infection could be reversed by gibberellic acid (Table 4).

Table 4

Reverse of dwarfing effect of systemic virus infection on Xanthi tobaccos*

Treatment	Stem length (cm)	Leaf weight (g)
Indol-3-acetic acid (10 ppm)	17.2	61.3
3-Indolyl-acetamide (10 ppm)	14.7	57.6
5-Hydroxy-indole-3-yl-acetic acid (10 ppm)	14.2	59.6
Gibberellic acid (GA_3) (10 ppm)	20.4	72.0
Benzyladenine (30 ppm)	12.9	41.5
Non-infected control	21.8	85.7
Infected control	14.4	59.2

* Measurement a month after inoculation with cucumber mosaic virus (Averages from 20-20 plants)

The indoleacetic acid increases the weight of plants only in a small extent and was not effective enough in reversing of dwarfing. The other hormons proved to be ineffective. The exogenous indoleacetic acid has a slight effect because one part of it becomes bound (cf.: ANDREA E and GOOD, 1955), on the other hand, as a substrate of indoleacetic acid peroxidase induces the formation of this enzyme (GALSTON and DALBERG, 1954).

The results achieved with gibberellic acid are in accordance with earlier observation of KURAISHI and MUIR (1962). Gibberellin treatment increased the endogenous auxin level of plants. In addition, SÁGI (1972) detected a lower activity of indoleacetic acid peroxidase in the cell-wall fraction of gibberellin treated plants. In this way the stunting effect induced by a virus infection can be controlled by gibberellic acid. MARAMOROSCH (1957) and ORLOB and ARNY (1961) similarly reversed dwarfing in plants infected by virus or mycoplasma. Our experimental results may refer to the reversing mechanism of virus-induced dwarfing effect.

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