

Increased Free Formaldehyde Level in Crude Extract of Virus Infected Hypersensitive Tobaccos

By

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The presence of free formaldehyde in pressed juice and extracts was detected by mass spectrometric measurement and by TLC in form of dimedone complex. An increased level of formaldehyde was found in the extract of TMV infected hypersensitive tobaccos as compared to the healthy control.

The presence of free formaldehyde in homogenates of different animal tissues was detected (KNECHT, 1966; PAIK and KIM, 1974) as a product of demethylation of N-methylated substances, e.g. dimethylnitrosamine (TURBERVILLE and CRADDOCK, 1971). The present paper reports on preliminary experiments carried out to demonstrate the occurrence of free formaldehyde in extracts of healthy and tobacco mosaic virus (TMV) infected hypersensitive tobacco leaves.

Nicotiana tabacum cv. Xanthi-nc was used in 6–8 leaf stage and was inoculated with U₁ strain of TMV. All inoculated leaves produced local necrotic lesions (hypersensitive necrosis) on the sites of virus infection. For direct detection of formaldehyde the leaf was directly applied to the double focusing mass spectrometer (JEOL JMS-01 SG-2). The samples of healthy and virus infected leaves were inserted into glass capsules and were taken into the electron impact ion source of the instrument. By slowly raising the temperature (5°C per min) from 20°C to 80–100°C, the spectrum of the evaporating components was taken in 8 or 16 sec interval. Fifty mass spectra were recorded from each sample to be able to check the mass fragmentation. (The intensity of ionizing current was 200 μA, ionizing voltage was 75 V, accelerating voltage was 10 kV, detector voltage: 1.8 kV, resolution power: 2000 and 5000.) During the mass spectrometric experiments the appearance of CHO⁺ and CH₂=OH⁺ (m/e = 29 and 31) ions and the alteration of their intensity were checked. The N₂⁺ ion (m/e = 28) served as comparative basis (100%). By the investigation of healthy and virus infected leaves the intensity of ions with mass numbers 29 and 31 increased from the 4th–5th scans, but in all cases of virus infected samples we got higher values of the ion intensities. The relative intensities of the peaks at masses 29 and 31 were the following:

	m/e = 29	m/e = 31
Healthy	34.0%	22.1%
TMV infected	138.0%	62.2%

These results demonstrated the occurrence of free formaldehyde both in healthy and virus infected tobacco tissues inserted into the glass capsules, but its amount significantly increased in TMV infected necrotic tissues.

Furthermore we homogenized leaf tissue in water, or acetic acid (5%) + trichloroacetic acid (5%) (30 g in 100 ml) and then, after centrifugation (5000 rpm for 10 min), 50 mg dimedone (5,5-dimethyl-1,3-cyclohexanedione) was added to the about 100 ml clear supernatant, and was preserved overnight. Then the precipitate was collected by centrifugation and was resolved in 2 ml chloroform. Samples of 0.05 ml were taken into silica gel G layers. The location of dimedone-formaldehyde adduct (bis-condensation product) on layer was identical with authentic substance (co-TLC) in two solvent systems (benzene-ethylacetate, 95 : 5 v/v; and chloroform-ethylacetate, 90 : 10 v/v. R_f values: 0.6-0.65 and 0.75-0.80, respectively). The spots were detected by the $FeCl_3 - K_3Fe(CN)_6$ reagent; Folin-Ciocalteu reagent; vanillin-ethanol reagent or 2,6-dichlorochinone-chlorimide (Fig. 1). The later reagent gave very specific color

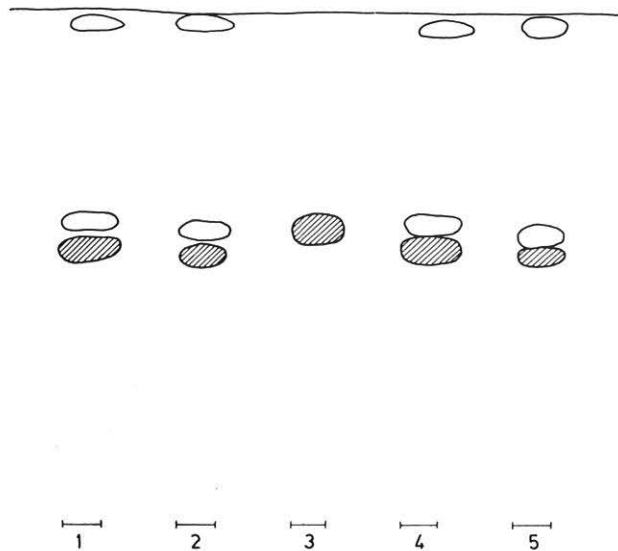


Fig. 1. TLC chromatography of formaldehyde in formaldemethone form in water extract of healthy and tobacco mosaic virus infected hypersensitive tobacco leaves. Layer: silica gel G (Merck); solvent: benzene - ethylacetate 95 : 5 v/v. Reagents: 0.2 g 2,6-dichlorochinone-chlorimide solved in 100 ml methanol, then 20 g of Na_2CO_3 solved in 100 ml water. Patterns: 1 virus infected; 2 healthy; 3 formaldemethone (10 μ g); 4 virus infected + formaldemethone (5 μ g); 5 healthy tobacco (15 g samples). Hatched spots = formaldemethone; empty spots = unidentified material

reaction with this dimedone-formaldehyde adduct (green in light and red in UV₃₆₅ light). The results of TLC experiments were strengthened by the mass spectrometrical investigations. After thin-layer chromatographic investigations the amount of free formaldehyde in healthy tobacco tissues is between 15–20 ng per g tissue. The presence of formaldehyde was detected both in water and in acetic acid (5%) extracts. But it was absent in the homogenate in trichloroacetic acid (5%). These results demonstrated that the observed formaldehyde was a product of enzymatic reaction, eventually of N-demethylase. The presence of trichloroacetic acid inhibited this enzymatic activity.

In the preliminary investigations of systemically infected tobaccos (*Nicotiana tabacum* var. *Samsun*) in compatible host–parasite relation we did not find any changes in free formaldehyde levels as compared to the control.

By these experiments we firstly verified the presence of free formaldehyde in extracts of tobacco leaf tissues and its increase in the virus induced hypersensitive necrosis (incompatible host–parasite relation). The role of increased formaldehyde production during the necrogenesis remains to be determined.

Literature

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