POST-INFECTION CHANGES IN LIPIDS OF THE ROOTS OF A COTTON PLANT VARIETY MODERATELY RESISTANT TO Verticillium dahliae

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UDC 633.511.581.4:632.4:547.915

A comparative study has been made of the lipid composition of healthy roots of the cotton plant of the Tashkent-1 variety (I) and of the corresponding plants infected with Verticillium dahliae Kleb. (II) grown under field conditions. The action of the factors of infection stress and seasonal and climatic conditions change the lipid metabolism. In lipids (I) and (II) seven classes of neutral lipids (NLs), three classes of glycolipids, and six classes of phospholipids (PLs) were identified and their fatty acid compositions were determined. It was established that in (II) there was a smaller amount of total lipids, NLs, and PLs, and an increased level of polyphenolic pigments, but the level of gossypol pigments with free aldehyde groups was unchanged. In the NLs of (II), as compared with (I), there were more triacylglycerols, sterols, triterpenols, and squalene. Complex changes were observed in the fatty acid composition of the acyl-containing lipids.

The penetration of the fungus Verticillium dahliae Kleb., into the tissues of the root system of the cotton plant is the initial stage of the interaction of the parasite with the plant. In experiments with isolated cotton-plant roots of the moderately resistant variety Tashkent-1 it has been shown that the parasite inserts itself into the cells and develops in them over the whole length of a root. In infected roots a suppression of synthetic processes and an increase in the level of phenolic compounds on the surface and the layers of the tissues have been observed [1].

We have made a comparative investigation of the lipids of the roots of healthy (I) and verticillium-wilt-infected (II) cotton plants of the Tashkent-1 variety grown under field conditions. There is no information in the literature available to us on the lipid composition of cotton-plant roots.

The yields of purified lipid extracts from roots (I) and (II) were 0.7 and 0.5% (on the weight of ground tissues). The extracts had a dark brown coloration and contained 17.12% (I) and 17.33% (II) (on the weight of the extracts) of free gossypol.

According to the literature [2], the main pigments of cotton-plant roots are polyphenolic compounds of gossypol nature, the high level of which in our samples interfered with analysis. It is known that by chromatographic separation on polyamide (PA) it is possible to separate the neutral lipids (NLs) of the cotton plant from a mixture of gossypol and gossypol-like pigments (GPs) with polar lipids [3], and we therefore attempted to employ this method. The extract was separated on a column of PA by using suitable solvents [4] into five pigment-colored fractions: 1) NLs + GPs; 2) NLs + glycolipids (GLs) + GPs; 3) GLs + GPs; 4) GLs + phospholipids (PLs) + GPs; and 5) PLs + GPs. The fractions were subjected to further purification: 1 and 2 were treated with pentane in which the NLs and, partially, the GPs dissolved while the other components precipitated; 4 and 5 were reprecipitated from acetone in which the GLs and GPs dissolved while the PLs formed the precipitate. In this way it was possible to separate the PLs from pigments almost completely. The fractions containing the GLs were combined and treated with a 2% solution of $NaHCO_3$, which permitted the partial separation of the GPs. However, it was impossible to achieve the complete separation of the NLs and GLs from pigments. Lipids of the same type were combined.

Institute of the Chemistry of Plant Substances, Uzbek SSR Academy of Sciences, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 59-63, January-February, 1989. Original article submitted March 29, 1988.

 0009-3130/89/2501-0050 $12.50

TABLE 1. Composition of the Neutral Lipids from the Roots of Healthy and Wilt-Infected Cotton Plants (% on the weight of the NLs)

Class	Sample I	Sample II
Hydrocarbons (HCs)		
1. C_nH_{2n+2}, C_nH_{2n}	7,3	Tr.
2. C_nH_{2n-2}, C_nH_{2n-4}	2,3	Tr.
3. C_nH_{2n-4}, C_nH_{2n-6}	4,5	Tr.
4. C_nH_{2n-6}, C_nH_{2n-8}, C_nH_{2n-10}	4,6	14,0
Fatty acid esters: with fatty alcohols with triterpenols with sterols with methanols	11,3	14,7
Triacyglycerols (TAGs)	18,9	17,6
Free fatty acids (FFAs)	13,4	10,2
Free triterpenols and sterols	14,7	19,1
Monoacylglycerols (MAGs) + chlorophylls	3,9	8,1
Gossypol pigments	13,3	10,6

The yields of the individual groups of lipids and fractions of gossypol pigments from (I) and (II) were as follows (% on the mass of the extract): NLs – 47.4 and 41.7; GLs – 18.1 and 19.1; PLs – 5.1 and 3.8; GPs – 28.8 and 35.4, respectively.

As we see, as a result of the infection the amounts of total lipids, NLs, and PLs in the roots had fallen while GPs had accumulated, but the level of gossypol pigments with free aldehyde groups had scarcely changed.

The NLs of the samples were separated into individual classes by column chromatography on silica gel. The classes were identified from their chromatographic behavior, by comparison with model samples, from the results of chemical transformations, and by their UV, IR, and mass spectra. The amounts of the individual classes were estimated gravimetrically after repurification and rechromatography. Information on the composition of the NLs is given in Table 1.

In the NLs of healthy roots, TAGs and hydrocarbons predominated (totalling 18.7%), while in the NLs with infected roots PAGs, free sterols, and triterpenols were predominant. Substantial differences were observed in the amounts of individual classes of NLs for the two samples, which reflected the complex metabolism of these compounds caused by the infection. Thus, in the lipids of the infected roots HC-1–3 had almost disappeared but the level of HC-4 had risen by a factor of 3, the amounts of MAGs and of bound and, especially, free sterols had increased, and the amount of FFAs had decreased slightly.

The hydrocarbon fractions 3 and 4 on treatment with 50% H_2SO_4 followed by heating gave pink colorations. HC-1 had in its UV spectrum λ_{max}^{hex} 228 nm and was not colored pink on treatment with 50% H_2SO_4.

In the mass spectrum of HC-1–4 the following ions were observed: M^+ 198-352, 420-436 (I); M^+ 262-360, 398-426 (2); M^+ 260-428 (3); M^+ 256-438 (4). To the strongest molecular ions, M^+ 198-240, 434, and 420 (1), M^+ 262-278 (2), M^+ 260-288 (3), and M^+ 256, 410 (4), corresponded to the fragments $M^+ - 15$, $M^+ - 43$, and $M^+ - 57$, and in fraction 4, also $M^+ - 69$. On the basis of a comparison of the chromatographic behaviors and mass spectra with literature information [5], the hydrocarbons were provisionally assigned to the C_nH_{2n+2} linear and isoprenoid alkanes and the C_nH_{2n} alkenes (1), to isoprenoids of the C_nH_{2n-2} and

C_nH_{2n-4} series (2) and to those of the C_nH_{2n-6} series (3) and to the C_nH_{2n-6}, C_nH_{2n-8}, and C_nH_{2n-10}, including squalene, $C_{30}H_{50}$ (M^+ 410) series (4).

Squalene predominated in the HCs of the lipids of the infected roots.

The esters were identified on the basis of the results of TLC and mass spectroscopy, as described in [6]. The maximum ions in the mass spectrum were those with M^+ 546, 544, 542, 533, 530, and 474, corresponding to $C_{37:2}$, $C_{37:1}$, $C_{37:0}$, $C_{36:2}$, $C_{36:1}$, and $C_{32:3}$ wax esters. M^+ 532 and M^+ 474 had the highest intensities. The spectrum contained fragments observed in the breakdown of esters of phytosterols and triterpenols. Of the fatty acids, 16:1, 18:1, and 18:2 residues were identified.

In the sum of the triterpenols and free sterols, the latter predominated. The GLC and mass-spectrometric analysis of the phytosterols showed that with respect to the qualitative set of components the samples were identical. The following were detected in them (%, GLC, for samples (I) and (II), respectively): β-sitosterol – 75.2 and 78.3; campesterol – 23.9 and 20.4; cholesterol – 0.9 and 1.3; stigmasterol – traces.

The chlorophylls gave absorption in the UV spectrum with $\lambda_{max}^{acetone}$ 660, 662 nm, corresponding to the types a and b.

In the glycolipids of the two samples by TLC in system 1 four spots were observed which were assigned to monogalactosyldiacylglycerols (MGDGs), R_f 0.86, digalactosyldiacylglycerols (DGDGs), R_f 0.74, and steryl glycosides, R_f 0.64. Visually, the main components of I and II were the MGDG's. The gossypol pigments were separated in the form of a smeared out spot from the start to the front of the plate with a concentration at the front, R_f 0.96.

In the PLs, with the aid of TLC (system 2) by comparison with model samples, we identified phosphatidylinositols (PIs), phosphatidylethanolamines (PEs), and phosphatidylcholines (PCs) and their lyso- analogs. The ratio of the main PLs within the total can be represented by visual estimation as the sequence PIs > PEs > PCs.

With respect to the quantitative set of the classes of PLs, samples (I) and (II) were identical. The quantitative amounts of the classes of GLs and PLs were not determined.

The total fraction of gossypol pigments and pigments accompanying the GLs gave with H_2SO_4 and with solutions of phloroglucinol and $SbCl_3$, positive reactions characteristic for free gossypol [7]. The pigments remaining in the NLs did not give these reactions. When the total gossypol pigments were separated on Silufol in system 3, three spots were detected with R_f 0.22, 0.4, and 0.66. The main spot was that with R_f 0.66 corresponding in its chromatographic mobility to a model sample of gossypol.

Attention is merited by the fact that on the separation of the cottonseed lipids on polyamide, the gossypol pigments were concentrated mainly in the GLs while in the case of the lipids of the roots the bulk of the GPs accompanied the NLs. No detailed analysis of the GPs was undertaken.

The compositions of the fatty acids in the acyl-containing classes of lipids determined by GLC are given in Table 2. The most considerable change in the total degree of unsaturation of the fatty acids was observed in the GLs, and it was less pronounced in the total lipids and the TAGs and MAGs, while in the FFAs and the PLs the total degree of unsaturation had, conversely, increased. The changes in the amounts of individual unsaturation of the acids in the lipids mentioned were not subject to the known tendency observed in the late blight Phytophthora infestans of the potato [8] and in a viral infection of the peanut [9] of a predominant fall in the level of the 18:2 species as a consequence of the more intensive peroxide oxidation of the lipids under infection stress. While in the PAGs this tendency was retained, in the MAGs and GLs the decrease in the amount of 18:1 was more pronounced than that of the 18:2 species. A possible explanation of this is the combined action on the metabolism of the root lipids of the factors of infection stress and the seasonal-climatic conditions of the field experiment.

EXPERIMENTAL

UV spectra were taken on a Hitachi instrument in hexane and acetone, and mass spectra on a MKh 1303 instrument at an energy of the ionizing electrons of 60/50 eV and a temperature of the ionization chamber of 120/80°C.

TABLE 2. Fatty Acid Composition of the Root Lipids of a Cotton Plant of the Variety Tashkent-1 (%, GLC)

Acid	Total lipids		Classes of lipids											
			TAGs		FFAs		DAGs		MAGs		GLs		PLs	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II
12:0	Tr.	0,5	—	0,3	0,5	0,4	1,4	0,7	0,2	0,5	0,2	0,3	—	—
13:0	Tr.	0,6	0,2	0,1	0,5	—	Tr.	—	Tr.	0,7	0,4	0,4	Tr.	0,2
14:0	0,6	0,9	0,2	0,4	0,6	1,0	0,7	0,4	1,0	1,3	1,5	1,9	0,5	0,3
15:0	0,8	2,2	0,1	0,3	0,2	0,3	0,7	0,4	1,5	1,5	1,2	2,3	0,6	0,4
16:0	31,0	29,3	15,4	17,4	40,4	34,0	26,8	28,5	30,8	30,4	44,3	54,7	48,1	44,7
16:1	0,9	1,2	1,4	1,4	2,1	2,5	2,4	2,4	4,4	2,6	0,9	1,8	2,8	4,4
17:0	0,7	0,9	0,2	0,5	1,2	1,2	1,2	0,7	1,2	1,5	0,9	2,4	1,0	1,7
17:1	0,9	0,9	0,2	1,0	1,8	1,8	0,9	0,4	0,6	1,0	1,3	—	0,2	0,7
18:0	3,9	4,4	2,4	2,0	4,8	6,0	4,2	4,0	5,2	6,0	11,4	17,5	2,7	3,7
18:1	28,4	26,1	41,0	45,6	37,7	36,9	39,5	35,1	23,7	20,8	21,0	6,0	10,0	8,7
18:2	20,4	20,2	26,3	20,8	8,3	12,2	17,1	23,8	26,8	25,1	8,9	2,8	22,1	26,4
18:3	9,4	8,2	12,6	10,2	1,1	3,4	5,1	3,6	—	—	1,0	—	12,0	8,8
20:0	3,0	4,6	—	—	0,1	—	—	—	4,6	3,8	1,1	3,6	—	Tr.
x_1	—	—	—	—	Tr.	—	—	—	—	—	1,6	—	—	—
22:0	Tr.	Tr.	—	—	0,3	—	—	—	Tr.	4,8	4,3	6,3	—	—
x_2	—	—	—	—	0,2	—	—	—	—	—	—	—	—	—
24:0	—	—	—	—	0,2	—	—	—	—	—	—	—	—	—
Σ_{sat}	40,0	43,4	18,5	21,0	49,0	43,2	35,0	34,7	44,5	50,5	65,3	89,4	52,9	51,0
Σ_{unsat}	60,0	56,6	81,5	79,0	51,0	56,8	65,0	65,3	55,5	49,5	34,7	10,6	47,1	49,0

*Trace amounts of the 10:0 acid were detected additionally in the TAGs and FFAs of samples I and II.

Gas-liquid chromatography was conducted as described in [6]. TLC on Silufol and silica gel was performed in the following solvent systems: 1) chloroform–methanol–water (65:25:4); 2) chloroform–methanol–ammonia (65:25:5); and 3) benzene–methanol (20:5).

The collection of the roots with the root colla from healthy and infected plants was made by workers of the laboratory for dwarf varieties of cotton plant of the G. S. Zaitsev Institute for the Selection and Seed Production of the Cotton Plant.

The gathered roots were dried for a week and were mechanically comminuted, and the lipids were extracted by Folch's method followed by their drying from water-soluble compounds on Molselekt [4]. The lipids were separated on polyamide as described in [4], and the NLs on silica gel as in [10].

The freeing of the GLs from the GPs with a 2% solution of $NaHCO_3$ was carried out by dissolving a sample in diethyl ether and extracting the solution three times with $NaHCO_3$ solution. The aqueous extracts were combined, a 10% solution of H_2SO_4 was added to give an acid reaction, and the gossypol that separated out was extracted with diethyl ether.

The freeing of the PLs from pigments was carried out by dissolving the fraction (50 mg) in chloroform (0.5 ml) with a few drops of methanol, and adding a solution of PLs dropwise to the cooled acetone (ratio of PLs to acetone 1:10). The PLs precipitated, the solution was centrifuged, and the mother liquor was separated by decantation.

The glycolipids were detected with α-naphthol, and the phospholipids with the Vaskovsky reagent, with ninhydrin, and with the Dragendorff reagent [11]. Sterol and triterpenol esters were revealed with 50% H_2SO_4 followed by heating to 150°C. Alkaline hydrolysis and the isolation of the fatty acids were carried out as described in [10]. The fatty acids were esterified with diazomethane. The amount of free gossypol was determined by a standard procedure [12].

SUMMARY

1. Seven classes of neutral lipids, three classes of glycolipids, and six classes of phospholipids have been detected in the lipids of cotton-plant roots, and their fatty acid compositions have been determined.

2. In the lipids of infected cotton-plant roots, as compared with healthy ones, the level of total lipids, neutral lipids, and phospholipids had fallen, and the total amount

of polyphenols had risen but the level of gossypol pigments with free aldehyde groups had not changed.

3. The changes in the amounts of individual unsaturated fatty acids had a complex nature.

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INFLUENCE OF WILT INFECTION ON THE GOSSYPOL PIGMENTS OF SEEDS AND ROOTS OF A COTTON PLANT OF THE VARIETY TASHKENT-1

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UDC 547.972

A study has been made of the gossypol pigments of the seeds and roots of a cotton plant of the variety Tashkent-1 infected with wilt in comparison with a healthy plant. The amount of gossypol in the infected plant was lower than in the healthy plant. In the diseased plant, gossypurpurin was concentrated in the roots, and in the healthy plant it was concentrated in the seeds. Gossypol possessing optical activity was detected in the seeds and roots of both the healthy and the diseased plants.

The resistance of the cotton plant to the causative agent of verticilliaceous wilt depends to a considerable degree on phenolic compounds and, above all, on gossypol, which participates in the protective reactions of the plant against attack by wilt [1].

We have made a comparative study of the gossypol pigments of the seeds and roots of a healthy cotton plant of the variety Tashkent-1 and of one attacked by verticilliaceous wilt.

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Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 63-66, January-February, 1989. Original article submitted April 22, 1988.

0009-3130/89/2501-0054 $12.50