

## EFFECT OF INFECTION WITH *Verticillium dahliae* ON THE BOUND AND STRONGLY BOUND LIPIDS OF COTTON SEEDS OF VARIETIES WITH CONTRASTING RESISTANCE

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*The lipids of the kernels of ripe cotton seeds of varieties resistant and susceptible to verticillium wilt have been determined. Intra- and intervariety changes in these indices for the infected plants have been revealed. Different directions of the post-infection changes in the lipids according to the degree of resistance of the variety to wilt have been shown. Differences have been observed both in healthy and in infected plants.*

There is information in the literature on the group, class, and fatty-acid compositions of the bound lipids of seeds of the wilt-resistant variety 108-F of the cotton plant *Gossypium hirsutum* L. [1], but there is no information on the amounts of bound glyco- and phospholipids in the seeds of the group composition of the native strongly bound lipids.

We have previously established that the kernels of ripe seeds of varieties resistant (175-F) and susceptible (Tashkent-1) to infection by *Verticillium dahliae* Kleb. differ with respect to their contents of lipids bound and strongly bound with protein (BLs and SBLs, respectively) [2]. Continuing these investigations, we have determined the group and fatty acid compositions of the BLs and SBLs of the seed kernels of the above-mentioned varieties of cotton plant and the change in these indices under the influence of wilt infection. The lipids from the seed kernels of healthy (sample I) and infected (sample II) of the two varieties were isolated as described previously [3]. The bound and the strongly bound lipids were separated by preparative TLC in systems 1 and 2 into groups of neutral lipids (NLs), glycolipids (GLs) and phospholipids (PLs). The results are given in Table 1.

It can be seen that in the BLs and SBLs of sample (I) of the resistant variety, the GLs predominated. In the susceptible variety the BLs contained a somewhat larger amount of PLs than GLs, while in the SBLs the amounts of NLs and PLs were identical. In the same sample from the resistant variety the amount of bound, but not of strongly bound, NLs was greater, while the amounts of PLs in the bound and strongly bound forms were 3.6 and 1.5 times lower than in the analogous sample from the susceptible variety. Thus, the SBLs of the kernels of healthy plants of the two varieties consist of the same groups of lipids as the BLs, but the ratio of NLs, GLs and PLs in them depends on the degree of resistance of the variety to wilt infection.

It can also be seen from Table 1 that, depending on the variety, the fungal infection had different effects not only on the amounts of BLs and SBLs but also on their group compositions. In the BLs of sample II from the resistant variety the amount of GLs had become even greater, but the level of NLs and, particularly, PLs had fallen in comparison with sample I. At the same time, in the BLs II of the susceptible variety the levels of all the groups of lipids were lower, although the fall in the amount of PLs was least. In the SBLs II of the resistant variety, as compared with SBLs I, the proportion of NLs had risen most considerably while the amount of PLs had not changed. In the susceptible variety the GLs had not changed while the PLs had fallen sharply.

Thus, in the lipids of the seeds of the resistant variety the GLs weakly and strongly bound with protein and the strongly bound NLs are the most important and the most variable in the quantitative respect, while in the seeds of the susceptible variety the PLs belong to this type of lipids.

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TABLE 1. Group Compositions of the Bound and Strongly Bound Lipids of the Seed Kernels of Healthy (I) and Wilt-Infected (II) Cotton Plants of the Two Varieties (%) on the a.d.m.)

Lipids	Neutral lipids		Glycolipids		Phospholipids	
	I	II	I	II	I	II
Bound						
175-F	0.65	0.43	1.24	1.41	0.31	0.16
Tashkent-1	0.31	0.18	0.98	0.57	1.11	1.05
Strongly bound						
175-F	0.22	0.54	0.35	0.53	0.23	0.23
Tashkent-1	0.35	0.32	0.30	0.30	0.35	0.18

TABLE 2. Fatty-Acid Compositions of the Bound Lipids of the Kernels of Healthy (I) and Wilt-Infected (II) Cotton Plants of Two Varieties (% , GLC)

Acid	Variety 175-F						Variety Tashkent-1					
	Neutral lipids		Glycolipids		Phospholipids		Neutral lipids		Glycolipids		Phospholipids	
	I	II	I	II	I	II	I	II	I	II	I	II
14:0	0.7	0.5	Tr.	Tr.	Tr.	0.3	Tr.	0.8	Tr.	0.9	Tr.	0.2
16:0	35.3	31.9	25.9	31.8	32.8	23.8	28.3	34.2	24.9	23.1	24.4	25.8
16:1	3.6	2.2	4.0	3.7	3.5	3.7	2.9	3.3	4.6	3.0	1.1	1.0
18:0	4.6	3.2	3.8	2.5	2.0	6.4	6.5	4.7	3.7	3.8	4.7	2.8
18:1	40.6	45.6	36.0	35.5	16.0	16.6	28.1	28.5	59.1	63.6	10.7	10.2
18:2	15.2	16.6	30.3	26.5	45.7	49.2	34.2	28.5	7.7	5.6	59.1	60.0
Σ <sub>sat.</sub>	40.6	35.6	29.7	34.3	34.8	30.5	34.8	39.7	28.6	27.8	29.1	28.8
Σ <sub>unsat.</sub>	59.4	64.4	70.3	65.7	65.2	69.5	65.2	60.3	71.4	72.2	70.9	71.2

These changes give grounds for assuming that the phytotoxic substances elaborated by the fungus *Verticillium dahliae* Kleb. affect the lipid compositions of the plasmatic and intracellular membranes, modifying their functions and, consequently, interfering with the cell metabolism.

The qualitative compositions of the BLs and SBLs of samples I and II of the two varieties did not differ, according to TLC, and consisted of known classes of nonpolar and polar lipids [1]. The fatty acid compositions of the lipids according to the GLC results are given in Tables 2 and 3. In addition to the components shown in Table 3, minor amounts of esters of the 19:0-26:0 high-molecular-mass acids were detected by spectrometry in the total methyl esters of the PLs of the susceptible variety, sample II.

As can be seen from Tables 2 and 3, the sets of fatty acids of the bound and strongly bound lipids from the healthy and wilt-infected cotton plants of the two varieties were similar. However, all the groups of SBLs of samples I differed appreciably with respect to their levels of individual fatty acids, particularly unsaturated acids, both between one another and between varieties. Thus, for the resistant variety the determining group of bound GLs was also more unsaturated than the NLs and PLs and contained almost equal amounts of 18:1 and 18:2 acids (Table 2). The bound PLs and GLs of the susceptible variety had similar high degrees of unsaturation of the fatty acids, but in the PLs the 18:2 acid predominated, and in the GLs the 18:1 acid. The neutral components of the SBLs of variety 175-F were enriched with the 18:1, and those of the variety Tashkent-1 with the 18:2, acid.

As a result of infection of the cotton plant with wilt, the changes in the quantitative levels of the fatty acids in the SBLs of the two varieties likewise took place differently. For the resistant variety the level of the 18:1 acid in the GLs increased more

TABLE 3. Fatty-Acid Compositions of the Strongly Bound Lipids of Seed Kernels of Healthy (I) and Infected (II) Cotton Plants of the Tashkent Varieties (%), (GLC)

Acid	Variety 175-F						Variety Tashkent-1					
	Neutral lipids		Glyco-lipids		Phospho-lipids		Neutral lipids		Glyco-lipids		Phospho-lipids	
	I	II	I	II	I	II	I	II	I	II	I	II
13:0	Tr.	2.7	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	2.2	1.8	Tr.	Tr.
14:0	1.9	3.2	1.1	2.3	0.3	0.9	2.2	1.7	0.8	2.8	0.9	1.1
15:0	1.6	Tr.	1.9	Tr.	2.2	2.8	1.5	1.5	2.2	2.5	1.8	3.4
16:0	40.2	36.1	59.8	57.4	40.0	48.8	39.2	39.1	61.7	50.2	35.2	50.4
16:1	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
17:0	4.0	2.6	Tr.	Tr.	2.0	1.8	2.9	2.9	Tr.	Tr.	2.1	Tr.
18:0	10.3	11.5	10.1	20.0	8.6	12.2	16.4	10.9	15.3	20.9	8.6	6.6
18:1	24.2	19.8	13.5	18.0	13.9	12.9	22.6	25.9	11.8	13.9	14.0	13.2
18:2	17.8	24.1	13.6	2.3	33.0	20.6	15.2	18.0	6.0	7.9	37.4	25.3
$\Sigma_{\text{sat.}}$	58.0	56.1	72.9	79.7	53.1	66.5	62.2	56.1	82.2	78.2	48.6	61.5
$\Sigma_{\text{unsat.}}$	42.0	43.9	27.1	20.3	46.9	33.5	37.8	43.9	17.8	21.8	51.4	38.5

appreciably, and there was less of the 18:2 acid in the GLs, while for the susceptible variety the levels of these acids in the NLs and GLs changed in the opposite directions. An exception was the bound PLs, the fatty acid composition of which in the two varieties scarcely changed after the infection of the plant. The principal saturated acid of all the bound lipids remained the 16:0 species, as for the free acids [1].

The degree of saturation of the total fatty acids of all groups of SBLs of the two varieties (Table 3) was 1.4-2.8 times higher than for the analogous groups of bound lipids, the most saturated being the GLs and, of them, the GLs of the susceptible variety, in which the amount of the 16:0 acid exceeded 60%.

In all the groups of SBLs appreciable amounts of the 13:0-15:0 medium-molecular-mass acids appeared, especially in the GLs of the variety Tashkent-1. Linoleic acid was concentrated in the strongly bound PLs and oleic acid in the NLs.

For the resistant variety, wilt infection was reflected most strongly on the composition of the fatty acids of the strongly bound GLs in the form of a sharp fall in the level of the 18:2 acid in them and an accumulation of the 18:0 and 18:1 acids. In the kernels of the susceptible variety the most pronounced changes due to infection were undergone by the fatty acids not of the GLs but of the strongly bound PLs, where, likewise, but to a less pronounced degree, the level of the 18:2 acid fell but the proportion of the 15:0 and 16:0 acids rose. Consequently, the quantitative fatty acid compositions of the individual groups of the BLs and SBLs of the samples under discussion also depended on the degree of resistance of the variety to the fungal pathogen.

## EXPERIMENTAL

The mass spectrum was taken on a MKh-1310 instrument at an ionization energy of the electrons of 60/70 eV and a temperature of the ionization chamber of 170/100°C.

GLC was conducted as described in [4].

Analytical GLC was performed on silica gel L-5/40 (Czechoslovakia) in the solvent systems: 1) chloroform; 2) acetone. The plates were revealed with iodine vapor and the lipids were detected with specific reagents [5].

The SBLs and SPBLs were isolated and nonlipid components were eliminated by the procedure of [3].

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