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EFFECT OF INFECTION BY *Verticillium dahliae* ON THE LIPID COMPLEX OF COTTON SEEDS FROM VARIETIES WITH CONTRASTING RESISTANCE

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The yields and fatty acid compositions of the free lipids and lipids weakly and strongly bound to proteins from seeds of cottonplant varieties resistant and susceptible to verticillium wilt have been determined and the changes in these indices in the seeds of the infected plants have been measured. It has been established that the seeds of healthy plants of the two varieties are similar in the qualitative compositions of the lipids and fatty acids but differ with respect the amounts of lipids in them bound with protein in different ways and with respect to the amounts of the individual fatty acids. The observed changes in the lipid and fatty-acid compositions depend on the degree of resistance of the variety to wilt.

In the seeds of oil crops, the lipids are present in states differing with respect to energy of the bonds with the protein of the kernel: in the mechanically retained state, in the sorbed state (free lipids — FLs), and in the states of chemical binding by hydrogen bonds (bound lipids — BLs) and by stronger covalent bonds (strongly bound lipids — SBLs) [1]. The free [2, 3] and bound [3] lipids of the seeds of certain varieties of cotton plant have been characterized. However, there are no comparative results in the change in the total complex of lipids of the cottonseed kernel under the influence of infection stress.

No investigations of the group and class compositions of the SBLs of the reserve tissues of plants have been made, although it is known that in animal tissues the lipid components strongly bound with protein in the form of lipoprotein complexes play an important role in the regulation of the immune system [4]. The compositions of the SBLs have been established for a number of such complexes, and it has been shown that the presence of certain lipids (phospholipids, triacylglycerols, free fatty acids) affects the degree of activity and the function of the lipoproteins.

We have previously [5] reported the isolation of the native SBLs from kernels of seeds of the cotton plant *Gossypium hirsutum* L., variety 175-F, which is resistant to infection by verticillium wilt *Verticillium dahliae* Kleb. Continuing these investigations we have made a comparative study of the complex of the lipids of the seed kernels of healthy (sample I) and wilt-infected (sample II) resistant and susceptible varieties (175-F and Tashkent-1, respectively) of the cotton plant.

The FLs, BLs, and SBLs were extracted successively from the ground kernels. The residual lipids (RLs), which, unlike the SBLs, were not liberated even with the aid of proteolysis, were extracted in the form of fatty acids (FAs) after alkaline hydrolysis of the protein residue under severe conditions. The yields of all the groups of lipids were determined after the separation of the nonlipid impurities (Table 1). Samples I of the two varieties scarcely differed with respect to the amounts of individual lipids, although it was possible to observe some predominance of the RLs in the susceptible variety and of FLs in the resistant variety. Infection lowered the level of the FLs of the two varieties more considerably than that of the other lipids. The fall in the level of the FLs of the resistant variety was higher than that of the susceptible variety.

The compositions of the SBLs of the samples under consideration were analyzed by TLC. The same components of the neutral and polar lipids of which the FLs and BLs were composed were detected [3]. The presence of polar lipids in the FBLs was confirmed by their IR spectrum.

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TABLE 1. Yields of Lipids from the Kernels of Healthy (I) and Wilt-Infected (II) Cotton Plants of Two Varieties

Lipids	Yield, % on the weight of the a.d.m. of the kernels (% on the weight of the lipids)	
	I	II
Free		
175-F	36.8 (92.9)	34.7 (92.3)
Tashkent-1	35.5 (91.9)	33.7 (93.0)
Bound		
175-F	2.2 (5.6)	2.0 (5.3)
Tashkent-1	2.4 (6.1)	1.8 (5.0)
Strongly bound		
175-F	0.8 (1.2)	1.3 (2.0)
Tashkent-1	1.0 (1.6)	0.8 (1.4)
Residual		
175-F	0.5 (0.3)	0.8 (0.4)
Tashkent-1	0.8 (0.4)	0.8 (0.6)
Total		
175-F	40.3 (100)	38.8 (100)
Tashkent-1	39.7 (100)	37.1 (100)

Thus, it has been shown for the first time that in the kernels of oil seeds, as in the nuclei of animal tissues [4], the free fatty acids, the triacylglycerols, and the phospholipids, and also the glycolipids, carbohydrates, and phytosterols, can form strong bonds with protein.

The sets of components of all the groups of lipids of samples I and II in the two varieties were identical.

The fatty acid compositions of the lipids isolated were determined by the GLC method. The fatty acids were represented by 10 components known for the lipids of cotton seeds. It had been shown previously that with an increase in the strength of binding of the lipids with protein the amount of the 16:0 acid in the total fatty acids increased [3]. The same feature is characteristic for the lipids of the samples under discussion.

In the acids of the SBLs and RLs, in addition to the components known for these lipids [3], we additionally identified the 13:0 and 15:0 acids and, in the RLs, the 12:0 acid as well.

The high content of the 14:0 acid in the SBLs and the RLs is not characteristic for the lipids of cotton seeds. We assume that the mixture of these acids obtained included an unidentified compound the retention time of which under GLC conditions coincided with that of the 14:0 methyl ester. On the subsequent separation of the SBLs into groups of lipids and analysis of their fatty acid compositions no increase in the amount of the 14:0 species was observed.

The intra- and intervariety changes in the amounts of the 18:2 and 18:1 unsaturated acids had a complex nature. In samples I and II of the two varieties the amounts of the 18:2 acid fell from the FLs to the SBLs, and in sample I of the resistant variety it fell further to the RLs, while also characteristic for this variety was a sharper fall in its amount (by 45%). In the RLs I of susceptible variety, this acid dominated. The highest level of the 18:1 acid was characteristic for the BLs I and II; with an increase in the strength of binding of the lipids with protein the proportion of the 18:1 acid in the mixture of fatty acids fell in all the acids except for the RLs of the resistant variety.

Thus, the seed kernels of healthy cotton plants of varieties resistant and susceptible to wilt have similar levels of FLs and SBLs with identical qualitative FA compositions but differ with respect to the amount of lipids strongly bound with protein (SBLs and RLs) which is 1.4 times greater for the susceptible variety, and also with respect to the amounts of individual acids in these lipids. As a result of wilt infection, the amount of FLs and BLs fell slightly, and the amount of SBLs and RLs

TABLE 2. Fatty-Acid Compositions of the Kernel Lipids of Healthy (I) and Wilt-Infected (II) Cotton Plants of Two Varieties

Acid	Free lipids				Bound lipids				Strongly bound lipids				Residual lipids			
	175-F		Tashkent-1		175-F		Tashkent-1		175-F		Tashkent-1		175-F		Tashkent-1	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
12 : 0	–	–	–	–	–	–	–	–	–	–	–	–	1.0	1.1	Tr.	0.5
13 : 0	–	–	–	–	–	–	–	–	0.2	3.0	0.3	0.4	Tr.	Tr.	Tr.	Tr.
14 : 0 + x	0.6	0.6	0.3	0.6	0.2	0.2	0.3	0.1	9.4	7.7	7.6	9.7	11.4	17.9	9.2	14.4
15 : 0	–	–	–	–	–	–	–	–	1.8	2.3	2.1	2.4	2.5	3.6	1.7	3.0
16 : 0	22.6	23.5	25.0	28.3	22.0	25.8	25.7	25.2	35.2	34.2	27.7	28.8	45.6	33.6	32.8	34.3
16 : 1	2.2	1.8	1.9	1.9	1.7	1.5	1.6	1.4	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
17 : 0	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0.7	1.8	0.6	1.5	Tr.	Tr.	Tr.	Tr.
18 : 0	2.3	2.3	1.6	0.4	2.8	2.8	3.0	1.9	7.0	7.1	5.7	5.6	7.2	5.7	3.3	2.5
18 : 1	15.6	17.1	17.2	18.6	26.8	28.3	29.5	29.1	19.1	20.6	18.5	20.4	20.7	13.6	12.5	13.7
18 : 2	56.7	54.7	54.0	50.2	46.4	41.4	39.9	42.3	26.6	23.3	37.5	31.2	11.6	24.5	40.5	31.6
$\Sigma_{sat.}$	25.5	26.4	26.9	29.3	25.0	28.8	29.0	27.2	54.3	56.1	44.0	48.4	67.7	61.9	47.0	54.7
$\Sigma_{unsat.}$	74.5	73.6	73.1	70.7	75.0	71.2	71.0	72.8	45.7	43.9	56.0	51.6	32.3	38.1	53.0	45.3

increases in the seed kernels of the resistant variety, while in the susceptible variety infection caused some decrease in the amount of all the groups of lipids except the RLs and a redistribution in the direction of an increase in the proportion of FLs and RLs.

Depending on the degree of resistance of a variety to wilt changes in the quantitative compositions of the fatty acids in different directions were observed in the seed kernels of the infected plants.

EXPERIMENTAL

IR spectra were taken on a UR-10 instrument in films. GLC was conducted as described in [8]. Analytical TLC was carried out as in [5].

The cotton seeds were provided by S. A. Usmanov and Yu. Ikramov of the G. S. Zaitsev Uzbek Scientific-Research Institute of Selection and Seed Production. The plants were grown in soil in a highly infected natural wilt zone. The infected plants were selected on the basis of the browning of the vessels of the stems observed on their longitudinal section, the necrosis of the leaves, and the number of deformed leaves.

The seeds were first freed from husks and the kernels were ground in a coffee mill.

The isolation of the FLs, BLs, SBLs, and RLs and the elimination of nonlipid impurities have been described previously [5].

IR spectrum of the SBLs (ν_{max}^{film}, cm^{-1}): 2960, 2930, 2860, 1460, 1350 ($CH_2CH_2CH_3$); 1740 (OCO); 1250 (−P═O); 1660 ($-NH_2$); 950 ($N(CH_3)_3$); 3200-3400 (−OH); 1110 (C−O−C).

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