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# PHOSPHOLIPIDS OF THE SEEDS OF THE HEALTHY AND THE WILT-AFFECTED COTTON PLANT

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A comparative study has been made of the phospholipid complexes of the seeds of a healthy cotton plant of the variety Tashkent-1 and one attacked by verticillium wilt. Some differences have been found in the amounts of the components of the total phospholipids and in the distribution of the fatty acids in the individual fractions of the phospholipids of the healthy and wilt-affected plants.

In a comparative investigation of the phospholipids (PLs) of the seeds of a healthy cotton plant of variety Tashkent-1 and one attacked by verticillium wilt, we have confirmed that there is a far smaller amount of gossypol in the seeds of the diseased plant. There are statements in the literature [1] that resistance to wilt is connected with the accumulation of gossypol in the cotton plant. Varieties resistant to wilt are attacked in time as a consequence of the broad possibilities of adaptation of the pathogen. These varieties actively react to the introduction of the parasite by an activation of various links of the metabolism. Bell [2] considers that the main fungitoxic substance in relation to the fungus is gossypol, and therefore a decrease in its amount in the seeds of the diseased plant may probably be connected with the consumption of gossypol in combatting the fungus.

On isolating the total PLs, it was found that their yield from diseased seeds was higher than from healthy seeds (1.8% as compared with 1.5%).

The qualitative and quantitative compositions of the total phospholipids were determined by two-dimensional thin-layer chromatography. It was established that the sets of PLs from the seeds of healthy and diseased cotton plants did not differ qualitatively from one another but there were differences in the quantitative distribution of the individual components in the total.

The quantitative distribution of the individual components in the total PLs of cotton seeds of the variety Tashkent-1 were as follows (% on the total PLS; $X_1$ and $X_2$ are unidentified PLs):

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| Plant | PCs | PIs | PEs | N-Acyl-PEs | Lyso-PCs | N-Acyl-lyso-PEs | $X_1$ | $X_2$ |
|---|---|---|---|---|---|---|---|---|
| Healthy | 53.5 | 19.3 | 16.2 | 4.0 | 3.2 | 1.4 | 1.4 | 1.0 |
| Wilt-affected | 38.0 | 22.0 | 22.0 | 5.6 | 4.9 | 3.9 | 2.0 | 1.6 |

A marked difference was observed in the amount of phosphatidylcholines (PCs) — there was a considerably smaller amount in the seeds of the diseased cotton plant than in the seeds of the healthy plant. There were more phosphatidylethanolamines (PEs) and phosphatidylinositols (PIs) in the seeds of the diseased plant. It is known that the phytotoxic substances produced by the fungus *Verticillium dahliae* Kleb. interact with the plasmatic and intracellular membranes, changing their function, and, consequently, disturbing the cell metabolism [3]. Many workers also consider that because of the disturbance of physiological and biochemical processes in diseased plants hydrolysis predominates over synthesis, as a result of which the metabolism is distributed [4]. Since PCs form the final product of the biosynthesis of PLs in plants, the decrease in their amount in the seeds of diseased plants is probably connected with a disturbance in the biosynthesis of the PCs (in the present case, possibly, from PEs) in the diseased plant.

The fatty acids (FAs) of the total PLs of the seeds of the healthy and diseased cotton plants were identified qualitatively (Table 1) and were also similar to one another quantitatively.

The individual fractions of the PLs were isolated by column chromatography on silica gel followed by preparative purification in a thin layer. On comparing the fatty acid compositions of the individual homogeneous fractions of PLs of the diseased and healthy cotton plants, it was observed that they were characterized in general by the same known laws, namely: position 2 of the phosphoglyceride molecules was predominantly occupied by unsaturated FAs; in the N-acylated PEs the saturated FAs, including capric, lauric, and myristic acids, were localized on the N atom to a greater degree. However, in spite of the retention of general laws, in the quantitative respect certain differences were recorded. In particular, in the PC fraction of the diseased cotton plant there was no stearic acid; the total degree of unsaturation was somewhat lower because of an increased content of palmitic acid. In the PEs of the diseased cotton plant there was a larger amount of unsaturated acids than in the PEs of the healthy plant, and in the PIs, because of the appearance of the $C_{10:0}$ and $C_{12:0}$ acids and an increase in the amount of stearic acid there was a larger amount of saturated FAs in the diseased plant. The N-acylated PEs of the diseased cotton plant were also more saturated than the PEs of the healthy plant. We must mention a difference in the amounts of capric acid: In the N-acyl-PEs of the diseased cotton plant its amount was fairly considerable and it was present wholly in the amide-bound form, while in the corresponding fraction of the healthy cotton plant there was none at all.

On comparing the position distribution of the FAs, it was noted that in the PC and PE fractions of the diseased cotton plant, position 2 was less unsaturated than in the healthy plant and in the PI fraction, on the other hand, a decrease in the degree of unsaturation in position was observed, i.e., it is possible to speak of a smaller specificity of the distribution of FAs in these fractions of the diseased plant. In the PE fraction of the diseased cotton plant, the $C_{10:0}$ and $C_{12:0}$ acids were present in position 2, and in the healthy plant in position 1. In the PIs of the diseased plant these acids were also concentrated in position 2 and in the PIs of the healthy cotton plant capric and lauric acids were absent.

## EXPERIMENTAL

The healthy and wilt-affected cotton plants were collected on an experimental plot of the Institute of Experimental Plant Biology, Academy of Sciences of the Uzbek SSR in 1978. The isolation of the total PLs, their separation into homogeneous components, and the investigation of these fractions were performed as described previously [5]. The quantitative distribution of the individual PLs was determined from the amount of phosphorus in the spots on two-dimensional TLC. The amount of phosphorus was determined by the Tevekelov method [6]. The alkaline hydrolysis of the PLs was carried out by a method described in Stahl's book [7].

For chromatography we used type KSK silica gel: 160-250 μ for column chromatography and up to 125 μ for thin-layer chromatography. The fatty acids were analyzed on a Khrom-41 gas–liquid chromatograph at 198°C in a column 2.5 m long filled with 17% of poly(ethylene succinate) on Celite-545.

TABLE 1. Fatty-Acid Compositions of the Total Phospholipids of the Seeds of Healthy and Wilt-Affected Cotton Plant of the Variety Tashkent-1 and Individual Fractions of Them

| Acid | Total phospholipids | Phosphatydylcholines | | | Phosphatydylethanolamine | | | Phosphatidylinositols | | | N-Acylphosphatidylethanolamine | | | N-Acyllysophosphatidylethanolamines | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | Position | | Total | Position | | Total | Position | | Total | O-Acyls | N-Acyls | Total | O-Acyls | N-Acyls |
| | | | 1 | 2 | | 1 | 2 | | 1 | 2 | | | | | | |
| **Healthy plant** | | | | | | | | | | | | | | | | |
| 10:0 | 0,4 | — | — | — | 2,5 | 3,0 | — | — | — | — | — | — | — | — | — | — |
| 12:0 | 0,3 | — | — | — | 2,2 | 2,4 | — | — | — | — | 4,1 | 1,5 | 5,2 | 1,5 | 1,0 | 9,6 |
| 14:0 | 0,7 | 1,2 | 1,9 | 1,7 | 1,8 | 2,4 | 1,4 | 2,4 | — | 2,4 | 3,9 | 1,5 | 5,0 | 1,0 | 3,6 | 7,0 |
| 16:0 | 25,0 | 18,2 | 35,1 | 3,2 | 22,1 | 49,2 | 4,6 | 31,9 | 52,6 | 6,0 | 25,2 | 28,2 | 39,8 | 23,6 | 22,3 | 23,0 |
| 16:1 | 1,2 | 1,7 | 2,4 | 1,7 | 3,8 | 2,4 | 1,9 | 3,7 | 9,4 | 3,3 | 5,2 | 2,2 | 4,3 | 1,3 | 2,5 | 5,2 |
| 18:0 | 3,9 | 3,2 | 5,9 | — | 4,1 | 2,4 | — | 5,6 | 9,5 | 3,1 | 4,6 | 2,0 | 3,5 | 3,9 | 1,9 | 5,5 |
| 18:1 | 17,7 | 23,1 | 21,9 | 23,8 | 12,5 | 4,5 | 14,4 | 10,4 | 11,1 | 9,2 | 12,9 | 12,1 | 17,6 | 21,1 | 13,3 | 17,9 |
| 18:2 | 50,8 | 52,6 | 32,8 | 69,6 | 51,0 | 33,7 | 77,7 | 46,0 | 17,4 | 76,0 | 44,1 | 52,5 | 24,6 | 47,3 | 54,9 | 31,8 |
| ΣS | 30,3 | 22,6 | 42,9 | 4,9 | 32,7 | 59,4 | 6,0 | 39,9 | 62,1 | 11,5 | 37,8 | 33,2 | 53,5 | 30,3 | 29,3 | 45,1 |
| ΣU | 69,7 | 77,4 | 57,1 | 95,1 | 67,3 | 40,6 | 94,0 | 60,1 | 37,9 | 88,5 | 62,2 | 66,8 | 46,5 | 69,7 | 70,7 | 54,9 |
| **Wilt-affected plant** | | | | | | | | | | | | | | | | |
| 10:0 | 0,1 | — | — | — | 1,7 | — | 3,4 | 4,6 | — | 1,5 | 5,2 | — | 18,8 | — | — | — |
| 12:0 | 0,1 | — | — | — | 0,8 | — | 3,4 | 3,4 | — | 1,8 | 3,2 | — | 11,6 | 1,2 | — | 8,8 |
| 14:0 | 0,6 | 1,3 | 2,5 | 1,2 | 0,6 | 1,7 | 3,5 | 3,9 | 2,3 | 1,2 | 2,2 | 2,3 | 6,8 | 2,4 | 1,8 | 5,7 |
| 16:0 | 23,1 | 25,1 | 42,3 | 15,9 | 24,8 | 69,2 | 5,4 | 25,3 | 64,7 | 3,5 | 27,0 | 31,1 | 22,6 | 30,3 | 26,1 | 33,8 |
| 16:1 | 0,9 | 1,8 | 3,8 | 1,1 | 0,8 | 1,5 | 2,6 | 4,5 | 2,5 | 1,3 | 4,4 | 2,7 | 7,1 | 3,3 | 2,3 | 4,1 |
| 18:0 | 5,1 | — | — | — | 1,5 | 3,0 | — | 8,0 | 12,9 | 1,9 | 7,9 | 3,9 | 8,2 | 3,9 | 3,1 | 4,2 |
| 18:1 | 18,6 | 19,6 | 19,1 | 20,3 | 9,7 | 5,9 | 18,1 | 10,5 | 5,6 | 10,4 | 17,6 | 11,2 | 12,6 | 10,6 | 10,4 | 14,3 |
| 18:2 | 51,5 | 52,2 | 32,3 | 61,5 | 60,1 | 18,7 | 63,6 | 39,8 | 12,0 | 78,4 | 32,5 | 48,8 | 12,3 | 48,3 | 56,3 | 29,1 |
| ΣS | 29,0 | 26,4 | 44,8 | 17,1 | 29,4 | 73,9 | 15,7 | 45,2 | 79,9 | 9,9 | 45,5 | 37,3 | 68,0 | 37,8 | 31,0 | 52,5 |
| ΣU | 71,0 | 73,6 | 55,2 | 82,9 | 70,6 | 26,1 | 84,3 | 54,8 | 20,1 | 90,1 | 54,5 | 62,7 | 32,0 | 62,2 | 69,0 | 47,5 |


## SUMMARY

A comparative study has been made of the phospholipid complexes of the seeds of a healthy cotton plant of variety Tashkent-1 and a plant of the same variety attacked by verticillium wilt.

It has been shown that the total amount of phospholipids in the seeds of the wilt-affected cotton plant is higher than in the seeds of the healthy plant.

It has been established that when a cotton plant is attacked by wilt substantial changes take place in the composition of the classes of phosphilipids: the amount of phosphatidylcholines in the total PLs decreases considerably.


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