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COMPARISON OF SOME SECONDARY METABOLITES IN COTTON LEAVES THAT DIFFER IN GENETIC MARKERS

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The change in the content of sterols, tocopherols, and polyprenols during three phases of cotton ontogenesis that differ in genetic markers was studied. The content of these components of self-marking strains and their parental varieties is compared.

Key words: α-tocopherol, sterol, polyprenols.

Plant growth regulators have a specific physiological effect on the hormonal system. Their activity is closely related to hormonal regulation and functions of the genetic apparatus. Therefore, it seemed interesting to study the composition of cotton leaves genetically modified for self-regulated growth and development.

We studied leaves of the cotton strains characterized below. The control was variety Tashkent-1 [1-3].

Strain L-249 is self-marking. Depending on the conditions of the year during the formation of 12-16 nodes, the point of plant growth stops functioning and, as a result, 2-5 valuable bolls are formed.

Strain L-4 is deciduous and self-marking. Genes responsible for growth are blocked during accumulation of 12-16 sympodialic branches. Strain L-4 was produced by hybridization of the early-ripening variety AN-Chelyaki-1 with the late-ripening rapid-growing strain L-598 and subsequent selection for deciduousness and self-marking. The vegetative period lasts for 125-135 days. The yield is 3800-4500 kilogramme/hectare (kg/ha). The deciduousness varies from 60 to 75%.

Early-ripening strain L-446 was produced by separating a population of variety AN-Chilyaki-1. The vegetative period lasts 100-115 days. The yield is 3500-4500 kg/ha.

Ultra-fast-ripening variety AN-Chelyaki-1 was produced by separating a varietal population of variety Tashkent-1. The vegetative period lasts 97-110 days. The yield is 2500-4400 kg/ha depending on the conditions of the year. The leaf surface is two times smaller than for Tashkent-1.

L-15-Ultra-Fast-Ripening Strain. Genes responsible for growth are blocked and genes controlling development are activated when the main stem reaches 70-80 cm in height.

Strain L-598 was produced by separating a varietal population of Tashkent-1 under continuous illumination. The L-598 bush is pyramidal. The vegetative period lasts 150-170 days. The yield is 3000-3800 kg/ha.

Variety Tashkent-1 [3]. The vegetative period is 135-145 days. The yield is 3500-4500 kg/ha depending on the conditions of the year.

Variety AN-306 of the species *Gossypium hirsutium* L. was produced by hybridization of variety Express-2 with the wilt-resistant deciduous Mexican variety A-3945. The AN-306 bush is compact. The vegetative period lasts 120-130 days. The yield is 3500-4500 kg/ha. AN-306 is a solid V type of the first industrial variety.

These features of the self-marking L-249 and L-4 and early-ripening strains L-446 and L-15 are important from the viewpoint of economics because they prevent mechanical marking. As a result the yield increases by 400-600 kg/ha. The dose of applied defoliants decreases because of the early-ripening and deciduousness of strain L-4.

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TABLE 1. Content of Principal Secondary Metabolites in Leaves of "Self-Marking" Early Ripening and Parental Cotton Varieties During Ripening in % of Total Unsaponified Substances

Principal components	Tashkent-1	AN-Chilyaki-1	L-598	L-249	L-446	AN-306	L-4	L-5
Sitosterol	6.0±0.6	8.5±0.85	8.7±0.87	8.6±0.86	8.6±0.86	8.7±0.87	8.5±0.85	7.6±0.76
Stigmasterol	3.1±0.31	3.1±0.31	4.19±0.42	4.1±0.41	4.1±0.41	4.2±0.42	4.4±0.44	4.1±0.41
α-Tocopherol	0.27±0.03	0.38±0.04	0.28±0.03	0.36±0.04	0.42±0.04	0.54±0.054	0.37±0.04	0.30±0.03
Polyprenols	52±5.2	77±7.7	64±6.4	85.6±8.6	86.6±8.7	87.2±8.7	86.6±8.7	76.7±7.7

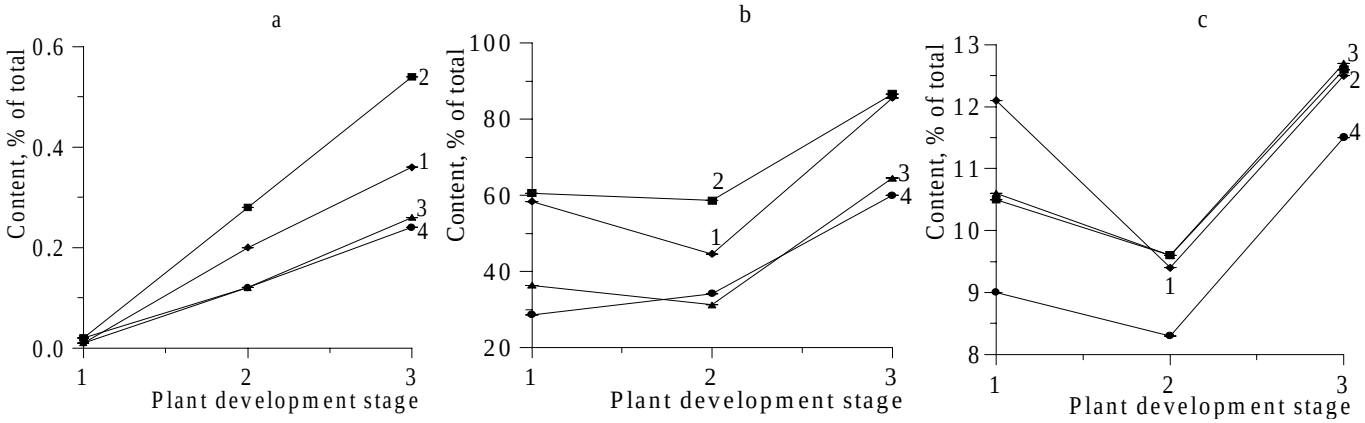

Fig. 1. Change of content of α-tocopherol (a), polyprenols (b), and sito- and stigmasterol mixture (2:1) (c) during phases of cotton development: L-249 (1), L-446 (2), L-598 (3), T-1 (4).

We have previously studied the composition and inhibitory activity of a fraction of secondary metabolites from cotton leaves of strain L-249 [4, 5]. Interesting data were obtained. Therefore, we decided to compare the composition of analogs and parental varieties and types during development phases in order to study the dependence of the content of secondary metabolites from autonomous growth regulation of the main stem.

Fractions were prepared according to the literature method [6].

The principal components of these fractions are α,β-tocopherols (oxidized forms of tocopherols depending on the cotton ontogenesis), polyprenols, and phytosterols [4, 5]. The change of content was studied by isolating fractions from leaves of these cotton strains during three development stages, i.e., formation of 2-3 actual leaves, budding—flowering, and ripening. Quantitative analysis was performed by quantitative TLC according to the literature [7].

The results are presented in Fig. 1 (a, b, c) and indicate that the amount of tocopherols in all studied strains increases as the plant develops. The maximal content occurs during ripening. However, the amount of polyprenols, the principal components of the fraction, decreases slightly during budding—flowering and reaches a maximum during ripening. The content of phytosterols changes similarly to the polyprenols.

A comparison of the content of the studied components in the self-marking strains shows that the amount of α-tocopherol, polyprenols, and phytosterols is greater than for the control Tashkent-1. The rapid-growing strain L-598 is intermediate in content of the principal component, polyprenols. The amount of tocopherol places it on almost the same level as the control Tashkent-1. The sterol content in this strain is slightly greater than in the self-marking strains.

The ability to transfer properties of parental varieties to the studied self-marking and early-ripening strains was studied by comparing the quantitative content of the components produced of these strains and the parental varieties An-Chilyaki-1 and L-598 during ripening, when the maximal content of the principal components was observed. Table 1 contains the results.

The contents of secondary metabolites in the leaves of the studied cotton varieties during ripening are significantly different. The minimal amount of sterols occurs in Tashkent-1, AN-Chilyaki-1, and L-15. The fraction of these components is nearly identical in the other strains. The early-ripening strains contain more polyprenols and α-tocopherol than the rapid-growing ones. It should be noted that the maximal amount of polyprenols and α-tocopherol is found in the industrial variety AN-306, in the self-marking and early-ripening strains, L-446. Therefore, it can be concluded that the parental variety AN-Chilyaki-1 transfers to self-marking and early-ripening strains its genetic property, namely, increased biosynthesis of tocopherols

and polyprenols; L-598, increased biosynthesis of sterols.

Thus, the content of sterols, tocopherols, and polyprenols depends on the cotton variety and strains. These components also play a decisive role in the autonomous regulation of plant growth. This is confirmed by their biological activity.

EXPERIMENTAL

Isolation of Fractions. Air-dried and ground cotton leaves (10 g, three ontogenesis phases) were extracted with ethanol. The concentrated extract was treated as before [6]. The yield of unsaponified fractions was 10, 10, and 10% of the air-dried mass of the plant for L-249; 7, 7, and 9% for AN-306; 8, 10, 9% for L-446; 10, 10, 10% for L-598; 9, 10, 10% for Tashkent-1; 10% (ripening phase) for AN-Chilyaki-1; 10% (ripening phase) for L-4; and 9% (ripening phase) for L-15, respectively.

The content of tocopherols, polyprenols, and phytosterols was determined by the literature method [7] and calculated using the formula:

$$\log W = \log W_s + [(A - A_s)/(A_d - A)]\log D,$$

where W is the mass of substance in the sample solution before dilution, W_s is the mass of substance in the standard solution, A_s is the area of the standard spot, A is the area of the sample spot, A_d is the area of the substance spot in the diluted sample solution, and D is the dilution coefficient.

Preparation of Standard Samples. Standard samples (10 mg) of α-tocopherol, polyprenols, and phytosterols were dissolved in distilled $CHCl_3$ (10 mL). Aliquots (1 mL) were diluted to 10 mL.

Rinsed Silufol-254 plates were used with elution by benzene—ethylacetate (24:1). The developers were Emmery—Engel reagent, H_2SO_4—vanillin (5%), and iodine.

The experiments were repeated three times. The relative uncertainty is 5-10%.

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