Chemistry of Natural Compounds, Vol. 33, No. 2, 1997

PHENOLIC COMPOUNDS OF THE PLANT *Gossypium hirsutum* AND OF CALLUS TISSUE FROM ITS ANTHERS

A. K. Karimdzhanov, N. N. Kuznetsova, and S. A. Dzhataev

UDC 547.972

The chemical composition of the catechins, leucoanthocyans, proanthocyanidins, and anthocyans of callus tissue of Gossypium hirsutum L. *has been studied in comparison with cotton plants growing under natural conditions. From callus tissue of lines L-29, L-32, and L-35 we have isolated (+)-catechin, (±)-gallocatechin, (−)-epicatechin, cyanidin 3-O-β-D-glucopyranoside, and cyanidin 3-O-[O-β-D-xylopyranosyl-(1→4)-β-D-glucopyranoside]. It has been shown that the components of the phenolic complexes in the plant and in callus tissue differ qualitatively and quantitatively.*

In culture, the tissues of some plant species (tea plant, tobacco, poppy, ginseng, rose-root stonecrop, mint, etc.) retain their capacity for synthesizing specific forms of natural compounds, including the polyphenols that are finding ever-increasing practical use [1-5]. Some of the main components of the cotton plant are phenolic compounds that are formed in the cotyledons and radicles during the first few day of the development of cotton seeds [6, 7]. The seed coats and boll valves and the bark of the stems and roots of the cotton plant contain various flavonols, catechins, leucoanthocyans, proanthocyanidins and anthocyans [8, 9]. The qualitative and quantitative compositions of these polyphenols in the various organs of the cotton plant change as the plant grows. At the beginning of the development of the plant, the simplest component of the polyphenols, (+)-catechin, is formed. In the cotyledons and radicles not only (+)-catechin but also (±)-gallocatechin, (−)-epigallocatechin, leucoanthocyans, anthocyans, and proanthocyanidins are formed.

Various parts of the cotton plant (stem, leaf, anther, immature embryo) introduced into culture on nutrient media with a definite composition give rise to the formation of callus tissue which retains the capacity for producing catechins, anthocyans, leucoanthocyans, and proanthocyanidins. For a study of the polyphenols of callus tissue, we used anthers of *G. hirsutum*, lines L-29, L-32, and L-35.

A comparative study of the phenolic compounds of the cotton plant and of a callus culture of this plant showed that the level of catechins and leucoanthocyans in the latter was lower than in the initial plant tissues. At the same time, in the tissue culture anthocyan pigments were synthesized in considerably larger amounts than the other polyphenols, and leucoanthocyans and proanthocyanidins in only very small amounts (see Table 1).

The difference in the ratios of the components of the phenolic complex in plants and tissue cultures is possibly connected with a weakening of differentiation and, partially, with a loss of the capacity for realizing genetic information relating to secondary metabolism.

Thus, with respect to the level, composition, and ratio of the individual components of the polyphenol complex, callus tissues of the cotton plant differ from various organs of the plant growing under natural conditions.

We are the first to have studied the phenolic compounds in a culture of the tissue of *G. hirsutum* anthers. It must be mentioned that the composition of the anthocyans and the shade of color of the pigment obtained from tissue cultures of line L-29, L-32, and L-35 were similar to those of the pigments of the cotton flowers.

A. S. Sadykov Institute of Biorganic Chemistry, Academy of Sciences, Tashkent, fax 62 70 71. Institute of Genetics of the Republic of Uzbekistan, Tashkent, fax (3712) 64 32 30. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 250-253, March-April, 1997. Original article submitted October 7, 1996.

0009-3130/97/3302-0187$18.00

TABLE 1. Comparative Levels of Phenolic Compounds in Various Organs of the Plant and in a Tissue Culture of Anthers of *G. hirsutum* (in % on the air-dry weight of the raw material)

Substance	Anther tissue culture			Organ of the cotton plant			
	L-29	L-32	L-35	boll valves	stem bark	root bark	flowers
(+)-Catechin	0.85	0.83	0.78	1.13	0.92	1.27	–
(±)-Gallocatechin	0.76	0.77	0.75	1.00	1.04	1.23	–
(−)-Epicatechin	0.12	0.10	0.12	0.15	0.22	0.30	–
(−)-Epigallocatechin	0.04	0.03	0.03	0.10	0.12	0.35	–
Chrysanthemin	3.60	3.37	3.34	0.05	0.10	–	2.20
Gossypicyanin	0.08	0.08	0.07	0.03	0.05	–	0.50
Leucocyanidin	0.01	0.01	0.02	0.41	0.51	0.57	–
Leucodelphinidin	0.01	0.02	0.02	0.46	0.48	0.59	–
Proanthocyanidins	0.20	0.03	0.04	2.60	2.42	3.40	–

Note. "–" means that this compound was not present or was detected only as a trace in the corresponding plant organ.

EXPERIMENTAL

Cultivation of *G. hirsutum* Anthers. We studied anthers of lines of a genetic collection of cotton plants created in the problem laboratory for the special genetics of the cotton plant at Tashkent State University [10]. Representatives of the lines L-29, L-32, and L-35 were characterized by the anthocyan coloration of the plants and had in their genotypes the dominant trait for anthocyan coloration (RpRp).

We used plants grown under natural conditions. Buds were isolated at the stage of mononuclear microspores (the stage giving the best results on cultivation) and were sterilized in 10% chloramine for 6-10 min and in 96% alcohol for 5 min, and then the anthers were isolated and placed in Petri dishes containing the nutrient medium. The whole process was conducted under sterile conditions, and the dishes were incubated at 28°C in a climate chamber in the light.

Explants with dimensions of 10-25 mg [sic] in groups of 10-12 were transferred to Petri dishes on standard agarized Nitsch–Nitsch medium with 3% of sucrose, the phytohormones 2,4-D and BAP – 1-2 mg/liter – and vitamins according to Gamborg.

Induction of a callus was observed on the 20th-26th day, and after 2-2.5 months the weight of the dark red callus had increased to 2.0-2.5 g. The callus tissues of the three lines were readily passaged on a medium of the same composition.

Isolation of the Catechins. The accumulated cottonplant callus tissues (400 g) were triturated in a porcelain mortar with 500 ml of 70% aqueous alcohol containing 0.1% of hydrochloric acid (extractant 1). The comminuted mass was transferred to a conical flask, covered with 1200 ml of extractant 1 and heated in the water bath under reflux at 40-45°C for 40 min. After cooling to room temperature, the extract was poured off and another 900 ml of extractant 1 was added to the flask. Extraction as described above was repeated. The combined extract from three such treatments was concentrated under vacuum to a dry matter concentration of 15% and, after preliminary treatment with chloroform, the catechins were extracted from the concentrated solution with ethyl acetate. The ethyl acetate extract was dried with sodium sulfate, filtered, and concentrated in vacuum. The total polyphenols (1.4 g) were precipitated from the concentrated ethyl acetate extract by the addition of a three- to four-fold volume of dry chloroform.

The polyphenols were separated and individual compounds were isolated by the method of [11]. The compounds isolated were (+)-catechin, (±)-gallocatechin, (−)-epicatechin, and leucoanthocyanidins. The leucoanthocyanidins were identified as leucocyanidin and leucodelphinidin by their conversion into the corresponding anthocyanidins – cyanidin and delphinidin [12].

Isolation of Chrysanthemin. After the extraction of the polyphenols, the anthocyans were isolated from the concentrated aqueous residue with *n*-butyl alcohol. The butanol extracts were combined and concentrated under vacuum. Precipitation of the anthocyan pigments from the concentrated butanol extract gave 0.24 g of total anthocyans. The anthocyans were separated by the method of [13], which led to the isolation of 0.15 g of chrysanthemin – cyanidin 3-O-β-*D*-

glucopyranoside. A second anthocyan, gossypicyanin – cyanidin 3-O-[O-β-D-xylopyranosyl-(1→4)-β-D-glucopyranoside] – was only detected chromatographically, since it was present in very small amount.

For analyzing the phenolic compounds we used paper chromatography, with the quantitative determination of catechins, leucoanthocyans, and proanthocyanidins [14]. The anthocyans were determined quantitatively by a method described previously [15].

REFERENCES

1. E. P. Kukhta, I. V. Alekandrova, V. N. Paukov, and M. A. Lyal'chenko, Khim. Prir. Soedin., 342 (1988)
2. N. G. Rybal'skii and O. M. Komarova, The Biotechnology of Polysaccharides [in Russian], VNIIPI, Moscow (1990), p. 3.
3. N. V. Zagoskina and M. N. Zaprometov, in: Abstracts of Lectures at the IVth All-Union Symposium on Phenolic Compounds [in Russian], Fan, Tashkent (1982), p. 33.
4. S. V. Lev, R. P. Zakirova, Z. Saatov, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 51 (1990).
5. D. A. Rakhimov and N. A. Shadmanova, Khim. Prir. Soedin., 440 (1994).
6. A. K. Karimdzhanov, A. I. Ismailov, and A. S. Sadykov, Khim. Prir. Soedin., 350 (1965).
7. A. S. Sadykov, A. K. Karimdzhanov, and Z. B. Rakhimkhanov, Dokl. Akad. Nauk UzSSR, No. 3, 36 (1961).
8. A. S. Sadykov, A. K. Karimdzhanov, and A. I. Ismailov, Uzb. Khim. Zh., No. 3, 60 (1962).
9. A. I. Ismailov, A. K. Karimdzhanov, Sh. Yu. Islambekov, and Z. B. Rakhimkhanov, Khim. Prir. Soedin., 3 (1994).
10. D. A. Musaev, Genetic Collection of Cotton Plants and Problems of Investigating Traits [in Russian], Fan, Tashkent (1979), p. 62.
11. M. N. Zaprometov and A. K. Karimdzhanov, Dokl. Akad. Nauk SSSR, **158**, No. 3, 162 (1964).
12. Sh. Yu. Islambekov, Kh. A. Mirzakhinov, A. K. Karimdzhanov, and A. I. Ishbaev, Khim. Prir. Soedin., 653 (1982).
13. A. S. Sadykov, Z. B. Rakhimkhanov, A. I. Ismailov, and A. K. Karimdzhanov, Nauchnye Trudy TashGU, No. 341; Khim. Prir. Soedin., 72 (1968).
14. M. N. Zaprometov, Fiziol. Rast., **5**, 296 (1958).
15. A. K. Karimdzhanov, Z. B. Rakhimkhanov, F. Kh. Mukhamedova, and A. I. Ismailov, Khim. Prir. Soedin., 382 (1995).