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PRODUCTIVITY OF *Ajuga turkestanica* CALLUS TISSUE DURING MULTIYEAR CULTIVATION

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Cell and tissue cultures retain the ability to produce substances that are specific for the original species. However, the biosynthetic potential often weakens during multiyear cultivation. This is evidently due to chromosomal changes. The frequency of chromosomal aberrations increases as the time of cultivation lengthens [1].

We studied the productivity of *Ajuga turkestanica* callus tissue (CT) during multiyear cultivation under standard growing conditions. We studied a culture of CT from ovaries of the plant [2] and strain 8-A, which was obtained after treatment with the mutagen N-nitroso-N-methylurea (N-NMU) at a dose of 8 mM for an hour [3]. Cultivation was performed in Murasighe—Skoog medium with saccharose (3%), α-naphthylacetic acid (1 mg/L), and thidiazuron (defoliant dropp) (0.02 mg/L) with transplantation every 4-5 weeks. Qualitative analysis was carried out by TLC on Silufol (Czech Rep.) plates using $CHCl_3$:CH_3OH (4:1). The amount of ecdysteroids was determined by a chromatography—spectrophotometry method that was developed earlier for determining ecdysterone in the whole plant [4]. Samples were extracted three times with MeOH and heating. A silica-gel plate (18×24 cm) was divided into four parallel bands. The extract of the studied samples was placed on two of these, a standard solution of ecdysterone on a third, and a blank sample on the fourth as a background run for spectrophotometry. Chromatography used $CHCl_3$—CH_3OH—$(CH_3)_2CO$ (6:2:1). Ecdysteroids in chromatograms were developed by spraying with vanillin (1%) in H_2SO_4. One of the bands with the sample solution and the blank band were developed.

The analysis showed that the productivity of *A. turkestanica* CT decreased during multiyear cultivation (Table 1). The yields of ecdysteroids decreased for both studied strains. Both ecdysterone and turkesterone were unstable. The CT derived from ovaries of the native plant produced in the first passage 0.1% ecdysterone and 0.032% turkesterone [2]. After one year of cultivation, the ecdysterone content was 0.04%; turkesterone, 0.007%. In the two-year culture, ecdysterone was 0.035%; in three-year, 0.030%; by the end of the fourth year, only traces were found. Turkesterone was detected in trace quantities in the two-year culture and was absent in three- and four-year cultures. Strain 8-A in the first passage typically had a high ecdysterone yield, 0.2%; turkesterone, 0.004%. The ecdysterone content decreased to 0.070% in the two-year culture; turkesterone, to trace quantities. Ecdysterone was 0.003% in four-year samples; turkesterone, not detected.

TABLE 1. Productivity of Two Strains of *Ajuga turkestanica* Callus Tissue

Cultivation time, yr	Original strain, %		Strain 8-A, %	
	Ecdysterone	Turkesterone	Ecdysterone	Turkesterone
3-6 passage	0.1	0.032	0.2	0.004
1	0.04	0.007	0.1	Tr.
2	0.035	Tr.	0.070	"
3	0.030	-	0.007	"
4	Tr.	-	0.003	-

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Thus, the yield of ecdysteroids during multiyear cultivation and the biosynthesis activity were found to decrease, evidently due to the predominance in the population of nonproductive rapidly growing cells.

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