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IN VITRO CULTURES OF *RHAMNUS FALLAX* BOISS. (RHAMNACEAE) AND ANTHRAQUINONE PRODUCTION.

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Rhamnus fallax Boiss., Rhamnaceae, is a shrub quite common in the mountain forests of south part of Serbia and Montenegro (J o s i f o v i ć 1973). The bark of this shrub contains anthraquinones (AQs) in the concentration of 6% of its dry weight (W i c h t l 1994; S t e i n e g g e r and H a n s e l 1988). This bark is used in folk and conventional medicine as a drug with laxative action. Furthermore, preparations with a standardized AQs content are made from this and other species of the Rhamnaceae family.

The leaves, bark and fruits of *R. fallax* were collected near the Brod Prizrenski village, Šara mountain, during August, 1995. Plant voucher is kept in the Herbarium of Botanical Garden, Faculty of Biology, University of Belgrade.

The seeds and zygotic embryos were isolated from the surface sterilized fruits. Zygotic embryos were then placed in the test tubes. The basal medium (BM) contained M u r a s h i g e and S k o o g (1962) mineral salts, 0.7% bacto-agar, 3% sucrose, supplemented with (in mg L^{-1}): 0.5 nicotinic acid, 0.4 thiamine (B1), 0.5 pyridoxine (B6), 2.0 glycine and 100 *myo*-inozitol. The pH of the medum was adjusted to 5.8 prior to autoclaving at 114 °C for 25 min. The tubes were placed at 25±2 °C, 16 *h* photoperiod, 2500 lux using cool white fluorescent tubes (Tesla, Pančevo). The seeds germinated after 7-10 days. The upper parts of seedlings were transferred to BM supplemented with 8.9 μM 6-benzylamino-purine (BAP) and 0.5 μM 2,4-dichloro-phenoxyacetic acid (2,4-D); the induction of buds was successful. Subsequently, to obtain material for propagation experiment, the shoots were grown in BM with the addition of 4.4 μM BAP and 0.5 μM indol-3-buteric acid (IBA).

For the study of *R. fallax* shoots propagation, different concentrations (0-20 μM) of BAP, kinetin (Kin) or thidiazuron (TDZ) with (0 - 10 μM) IBA, indol-3-acetic acid (IAA) and α-naphthaleneacetic acid (NAA) were added to BM. Axillary buds satarted to grow on the node segments within a few days. Later, the explants developed callus tissue on the contact surface with the medium and numerous adventitious buds were formed. The results show that 5 μM BAP and 0.5 μM IBA in BM was the combination suitable for micropropagation (Table 1). On this medium 5-6 vigorous shoots *per* explant were produced in four weeks.

The root induction on the plantlets was conducted with different concentrations (0-10 μM) of IBA, NAA and IAA in half strength BM. High percentage (70%) of *R. fallax* shoots were rooted in the medium containing 10 μM IBA. On the average, three short, thick and unbranched roots were formed per shoot grown in the medium mentioned above.

Table 1. Multiplication of *Rhamnus fallax* buds

Plant growth regulators		Concentration (μM)	Number of axillary buds	Number of adventitious buds
5 μM BAP	Control	0	3.1 ± 0.17	0.7 ± 0.06
	IBA	0.5	4.1 ± 0.33	1.0 ± 0.11
		1	4.3 ± 0.24	1.2 ± 0.12
		2.5	3.9 ± 0.34	1.3 ± 0.13
		5	3.9 ± 0.27	0.4 ± 0.12
		10	4.2 ± 0.36	1.0 ± 0.11
	NAA	0.5	1.4 ± 0.19	0.1 ± 0.03
		1	0.9 ± 0.16	0.2 ± 0.06
		2.5	0.5 ± 0.10	0.1 ± 0.03
		5	0.7 ± 0.10	0.2 ± 0.06
		10	0.6 ± 0.12	0.2 ± 0.06
	IAA	0.5	1.5 ± 0.15	0.2 ± 0.05
		1	1.4 ± 0.16	0.4 ± 0.08
		2.5	1.7 ± 0.13	0.5 ± 0.07
		5	0.6 ± 0.11	0.1 ± 0.03
		10	0.5 ± 0.11	0.0 ± 0.00
0.5 μM IBA	control	0	0.1 ± 0.02	0 ± 0.00
	BAP	1	2.2 ± 0.26	0.1 ± 0.05
		5	5.3 ± 0.28	0.7 ± 0.13
		10	4.4 ± 0.23	1.2 ± 0.18
		20	4.5 ± 0.31	1.2 ± 0.17
	Kin	1	0.1 ± 0.04	0.0 ± 0.00
		5	0.1 ± 0.06	0.0 ± 0.00
		10	0.7 ± 0.13	0.1 ± 0.05
		20	1.3 ± 0.20	0.2 ± 0.07
	TDZ	1	1.6 ± 0.14	0.8 ± 0.08
		5	1.7 ± 0.18	0.9 ± 0.08
		10	0.8 ± 0.11	0.5 ± 0.08
		20	0.4 ± 0.09	0.1 ± 0.04

*Values are means obtained from no less than fifty schoots in two consecutive passages

Table 2. Average quantities and composition of AQs in the different tissues of *Rhamnus fallax*

	Tissue	Total AQ aglyconse (%) x ± SE	Ratio between free and bound AQ aglycones	Ratio between main AQ aglycones (%)			
				ae	e	ch	ph
from nature	leaves	0.34 ± 0.03	1 : 5.7	17	41	31	11
	bark	1.35 ± 0.05	1 : 8.1	4	34	8	54
	unripe fruit	1.36 ± 0.10	1 : 1.8	62	22	15	1
	ripe fruit	0.38 ± 0.05	1 : 1	77	12	9	2
from *in vitro* culture	shoot	1.14 ± 0.25	1 : 1.9	19	19	29	33
	callus tissue	0.47 ± 0.06	1 : 2.6	18	42	19	21

21ac = aloe emodine, e = emodine, ch = chrysophanol, ph = phiscion

*Values are means of no less than six measurements

The callus tissue was initiated on the leaf petals when they were transferred to the BM supplemented with 2.2 μM TDZ and 0.5 μM NAA. This callus tissue, shoots of *R. fallax* (grown at 5 μM BAP and 0.5 μM IBA), as well as the leaves, bark and fruits of this shrub were analyzed for their production of AQs. It was confirmed by using Bornträger test (S t e i n e g g e r and H a n s e l 1988) that all examined tissues contained free and glycoside bound AQs. HPLC method (S a j c *et al.* 1999) was performed for quantitative analyses. The results are presented in Table 2. The shoots of *R. fallax* grown *in vitro* were good producers of AQs. They contained almost the same quantity of AQs as the bark of the shrubs grown in the field. This is important because the *Fallacis cortex* is a drug used for laxative action. Composition of metabolites synthesized by *in vitro* cultures was not different from that of the leaves and bark of intact plants. According to total content of AQ aglycones, callus and shoots of *R. fallax* were better producers of these metabolites than *in vitro* cultures of some other plants from the Rhamnaceae family (S a j c *et al.* 1999).

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