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ASPECTS CONCERNING HYDROQUINONE EFFECT ON CELL CULTURES OF *ARCTOSTAPHYLOS UVA-URSI*, *CATHARANTHUS ROSEUS* AND *DIGITALIS LANATA*

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Abstract: The experimental support for hydroquinone (HQ) bioconversion to arbutin consisted of cell cultures of: *Arctostaphylos uva-ursi* (6AUU cell line), *Catharanthus roseus* (Cr cell line) and *Digitalis lanata* (15 and 8HQ cell lines). HQ (6 mM) was added to the culture medium and displayed different effects on the physiological processes we had in view, such as: growth, cell viability and peroxidase activity. These processes varied depending on the species or cell lines, amount of fresh inoculum and duration of contact with HQ. Although cell growth suffered less significant variations, cell viability was strongly inhibited in all cell suspensions after the 6th day of culture. *D. lanata* cell lines showed the highest resistance to HQ and the most sensitive cell line proved to be *A. uva-ursi*. In *C. roseus* and *D. lanata* (15 cell line), IEF (isoelectric focusing) revealed a basic isoperoxidase (usually involved in stress response). This basic peroxidase had not been expressed in *Digitalis lanata* (8HQ cell line), which may explain it is higher resistance to HQ. Isoperoxidases associated with the growth process have been also identified. TLC (thin layer chromathography) revealed that all plant cell cultures biotransformed HQ to arbutin, excepting *A. uva-ursi* cell line.

Introduction

Cultured plant cells biotransform many kinds of substrates supplied exogenously via a wide range of reactions, such as glucosylation, glucosyl esterification, hydroxylation, oxido-reduction between alcohol and ketone, reduction of carbon double bonds, hydrolysis, isomerization, epoxidation, dehydrogenation, methylation, and others [7,19,23]. Among such biotransformation reactions, glucose conjugations (glucosylation and glucosyl esterification) are major reactions; they are the subject of 45% of published reports on biotransformation [23].

The *O-β-D-glucoside* of hydroquinone (HQ), also named arbutin, is a widely distributed compound in various higher plants, especially in species such as *Arctostaphylos uva-ursi* (L.) and *Vaccinium vitis-idaea* (L.) Sprengel [18]. From the farmacological point of view, arbutin has attracted much interest for two therapeutical applications. As it exhibits an antibacterial effect, the leaves of *Arctostaphylos uva-ursi* are used as tea preparations for the treatment of infections of the urogenital tract [21]. This glucoside is also a potent suppressor of biosynthesis of melanin in human skin [2].

One of the most promising procedures for biological arbutin production was the biotransformation in plant cell suspension cultures of exogenously added aglycone hydroquinone with resultant detoxification by glucosylation. Earlier experiments with plant cell culture systems clearly indicated such biotransformations but the yields were relatively low [14,20]. However, cultivated cells of the alkaloid-delivering Apocynaceous species of *Catharanthus roseus* (L.) G. Don, were proven to be able to produce up to 9 g of pure arbutin/l cell suspension [9], which was the highest production of a natural product by cultivated plant cells at that time. When a similar process was developed with a high density *Rauvolfia* cell suspension instead of the *Catharantus roseus*, the amounts of formed arbutin could be considerabily enhanced with an optimum

accumulation of 18 g/l nutrition medium [13], demonstrating the exceptional glucosylation potential of these cells.

Several reports have been published on differences in biotransformation ability of the diverse plant cell cultures. Yokoyama [23] considered a number of possibilities as factors affecting the ability of cell suspensions to mediate glucosylation: the plant species, the cell lines or strains, the age of the culture, the cell density, the concentration of the substrates etc. This aspect has been demonstrated for the HQ's bioconversion by plant cell cultures of *Catharanthus roseus* [10,26]. However the effect of HQ on the physiological processes we had in view was not reported so far [16].

In this paper our attention was focused on the ability of different plant cell cultures to biotransform exogenously added HQ to arbutin (A). We have investigated the effect of HQ on: cell growth, cell viability and enzymatic activity, in diverse plant species, cell lines and at different cell densities. Therefore two cell lines (15 and 8HQ) of *Digitalis lanata*, one of *C. roseus* and one of *Arctostaphylos uva-ursi* have been used. We had also examined by TLC (thin layer chromathography) the feasibility of these cell suspensions to efficiently produce arbutin by biotransformation.

Material and Methods

Cell cultures. The cell suspensions of *Cataharanthus roseus* and *Arctostaphylos uva-ursi*, established by Deliu et al. (Deliu, unpublished data), were grown in Murashige and Skoog (MS) [15] medium with 3% sucrose and 0.5 mg/l 2,4-D. For *Digitalis lanata*: the 15 cell line was cultivated on basal MS medium supplemented with 3% sucrose, IAA (1.0 mg/l) and kinetin (2.0 mg/l); the 8HQ (cell line habituated and HQ-resistant), was cultivated on basal MS medium supplemented with 3% glucose, but without phytohormones [4]. All cell suspensions were subcultured at every 14 days. The ratio between the volume of the inocula and the volume of the fresh medium was 1:6. Cell suspensions were grown in 300 ml Erlenmeyer flasks (with 40 ml liquid medium/vessel) on a gyratory shaker (100 rpm) and maintained in the dark at 25±1.5 °C.

Experiments with HQ. The cells at 9 day of culture stages were collected by filtration and were inoculated in the 100 ml flasks with 30 ml biotransformation medium. For *C. roseus* and *D. lanata* two different cell densities (expressed in fresh weight g/l) were used: I = 133.3 g/l (about 12%) and II = 233.3 g/l (about 17%). In the case of *A. uva-ursi* we tested only the 12%. Biotransformation medium for *C. roseus* and *A. uva-ursi* was MS with 6% glucose, 0.5 mg/l 2.4 D and 0.1 mg/l BA. The biotransformation media for the 15 and 8HQ *D. lanata* cell lines are a like to the growth media. The only parameter changed was the concentration of glucose: 6%. HQ was added from day 0 by sterile filtration. In each flasks, 1 ml of solution containing 19.8 mg HQ, was added (this quantity corresponds to a final concentration of 660 mg/l). Periodically at every second day (at 2, 4, 6 and 8 days), the cell suspensions were harvested and analyzed for a series of parameters: cell morphological traits, cell growth (fresh weight), cell viability, izoperoxidase activity and arbutin synthesis in cells and in medium. Arbutin production in the controls was tested only for the species *A. uva-ursi.* In its natural habitat this plant synthesizes normally arbutin. Thus it is fair to believe that the control cell suspension of this plant could have been synthesizing arbutin without requiring HQ addition as a precursor.

Cell viability. To measure cell viability we used an alternative technique based on TTC ability to color in red the viable cells, dead cells remaining uncolored [5,25]. Fresh cells (0.2 g) were washed with phosphate buffer (pH 7.0) and suspended in 3 ml solution of TTC (0.6% in phosphate buffer, pH 7.0) after incubation overnight in the dark at 26°C, the cells were analyzed at the microscope. For us to appreciate cell viability we counted the viable cells from a number of 500 cells.

Enzymatic activity. Electrophoretic separation of izoperoxidases has been performed by isoelectric focusing (IEF) on 5% polyacrylamide gel and ampholynes pH 3.5-5 and 3.5-10, 1:1.

The cell suspension material consisting of 100 mg fresh cell biomass was mortared, suspended in buffer containig glycerol, ampholynes, water, brom-phenol-blue. Samples were centrifuged 30 min at 10000 rpm. The runing buffer was 20 mM NaOH / 10 mM H_3PO_4. For izoperoxidases detection we used 3-amino-9-ethyl-carbazole and dimethyl-phormamide [1].

Determination of arbutin. Arbutin was extracted from dry weight (80 ºC, 24 h) of cell suspensions. The qualitative analysis of arbutin and HQ has been performed by thin layer chromatography (TLC) according to Stahl [17] method. Chromatographic plates: kieselgel GF_{254}. Mobil phase: chloroform: methanol (95:5). On the chromatograms arbutin is represented by a spot with $R_f = 0.46$ and HQ is represented by a brown spot with an $R_f = 0.88$.

Results and Discussions

Morphological aspects in experimented cell lines.

Morphological characterization of cell suspensions may offer some valuable information towards their different glucosylation capacity. Therefore we analyzed before each biotransformation experiment the morphological traits of every cell suspensions as in their normal growth medium, before the introduction of HQ (Fig.1A - D).

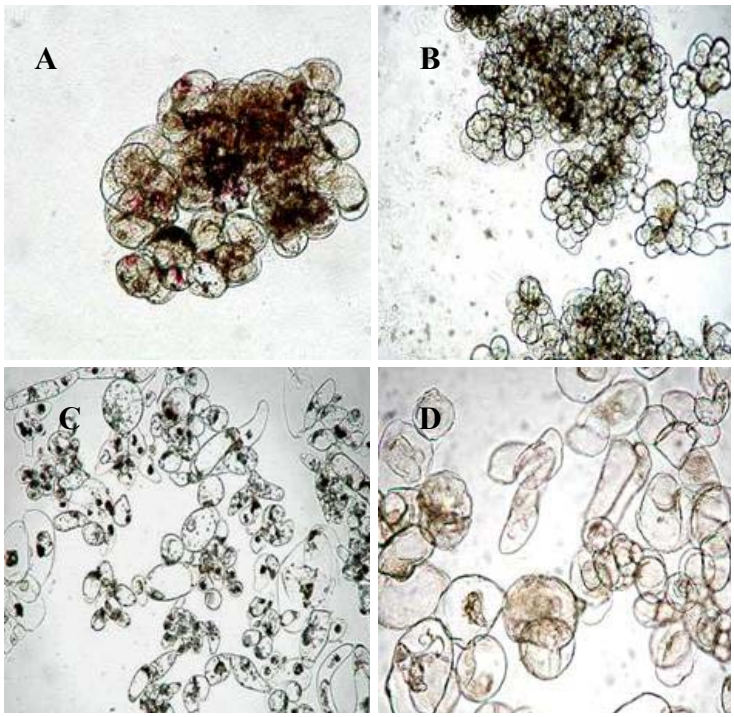

Fig. 1: Morphological aspects of cell lines (control) in growth media. A. 15 cell line of *Digitalis lanata*; B. 8HQ cell line of *Digitalis lanata*; C. Cr cell line of *Catharanthus roseus*; D. 6AUU cell line of *Arctostaphylos uva-ursi*

Plant cell suspensions of *Arctostaphylos uva-ursi* (Fig. 1D) consisted either of small globular cells or elongated cells with large vacuoles. Seldom smaller cell aggregates were found.

Examining *Catharanthus* (Fig. 1C) suspension culture only free cells were found. Some of them were globular, dense and smaller while others were cylindrical in shape with well developed vacuoles. The proportion between these two types of cells was in favor of globular cells with fewer and smaller vacuoles. This cell suspension proved to be the most exquisite as consistency.

Concerning the two different cell lines of *Digitalis lanata* (Fig. 1A and 1B) they consisted of large, round cells with larger vacuoles than in the case of *Catharanthus roseus*. Most of them were concentrated in cell aggregates; free elongated cells were very rarely detected. The cell aggregates proved to be larger in the case of 8HQ cell line (Fig. 1B).

Growth of cell suspensions in biotransformation medium.

The results were eloquent and related, with the morphological traits of cell suspensions, with the amount of fresh inoculum and with the concentration of HQ (Fig. 2A - D).

In this context, the best growth was recorded in controls, which had no HQ included in the biotransformation medium. For instance, 8HQ *D. lanata* cell line reached the highest growth for all suspensions at 8 days after the inoculation - 354g/l biomass (Fig. 2B). 8 HQ *Digitalis lanata* cell line showed the highest cell density and possesed the largest cell agregates (over 300 cells/agregate) consisting of both small, globular cells and large cells with well-developed vacuoles.

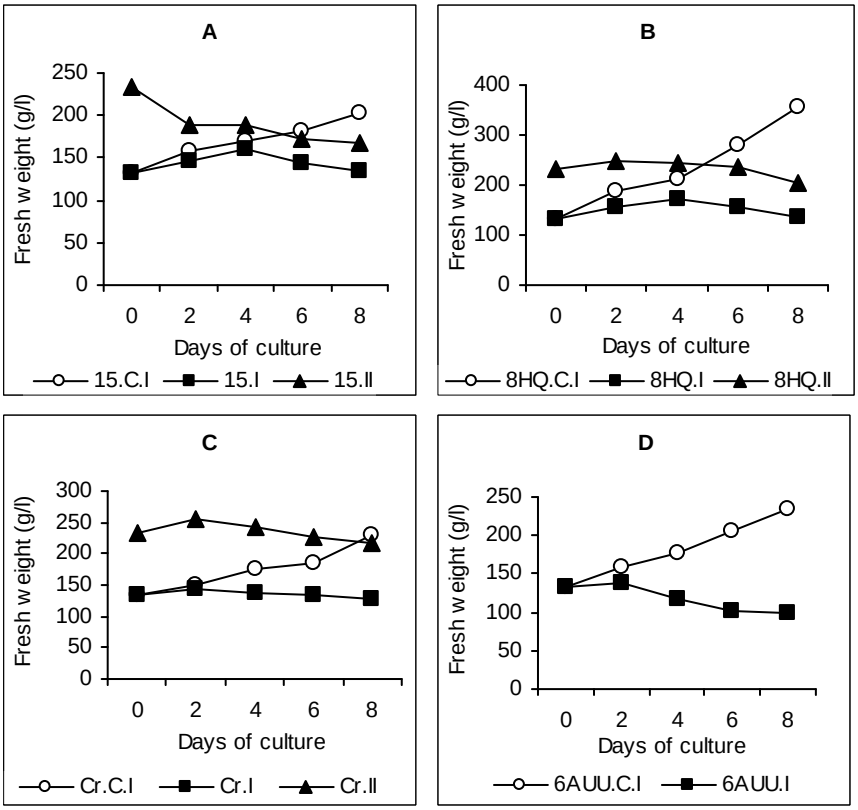

Fig. 2: Effect of HQ (660 mg/l) on cell growth of cell suspensions. A. *Digitalis lanata* (15 cell line); B. *D. lanata* (8 HQ cell line) ; C. *Catharanthus roseus* (Cr cell line); D. *Arctostaphylos uva-ursi* (6AUU cell line). C = control; Fresh weight of inoculum: I = 133 g/l; II = 233.3 g/l

Concerning the amount of the fresh inoculum, between the two biomass quantity used (I = 133.3g/l; II = 233.3g/l fresh wt.), we observed there less significant differences in decrease or increase of cell growth of the variants. The variants with lower fresh amount of the inoculum proved a faster growth in the first days after the inoculation than the variants with 233.3g/l amount of inoculum. The faster growth of lower cell density variants is normal because they had more available medium for growth than those cell suspensions with a higher cell density.

On the whole HQ inhibited cell growth in all employed cell lines. The intensity of the process showed variations depending especially on cell line (Fig. 2A - D). Thus, comparing to the control, cell growth was less affected in *Digitalis lanata* 15 cell line, in which after 8 days of incubation with HQ, the growth rate dropped with 34% (Fig. 2A). Accordingly, a higher

sensitivity to the added HQ was registered in the case of *Arctostaphylos uva-ursi* cell line, in which the inhibition of the process surpassed 58% (Fig. 2D).

Cell viability.

Cell viability dropped considerably with every second day. The major cause was the toxic effect of HQ on cultured cells. Lower cell density suspensions were the most affected because concentration of HQ experimented (660mg/l) proved to be very harmful for the amount of 133.3g/l fresh biomass. With every day arbutin decreased in these suspensions.

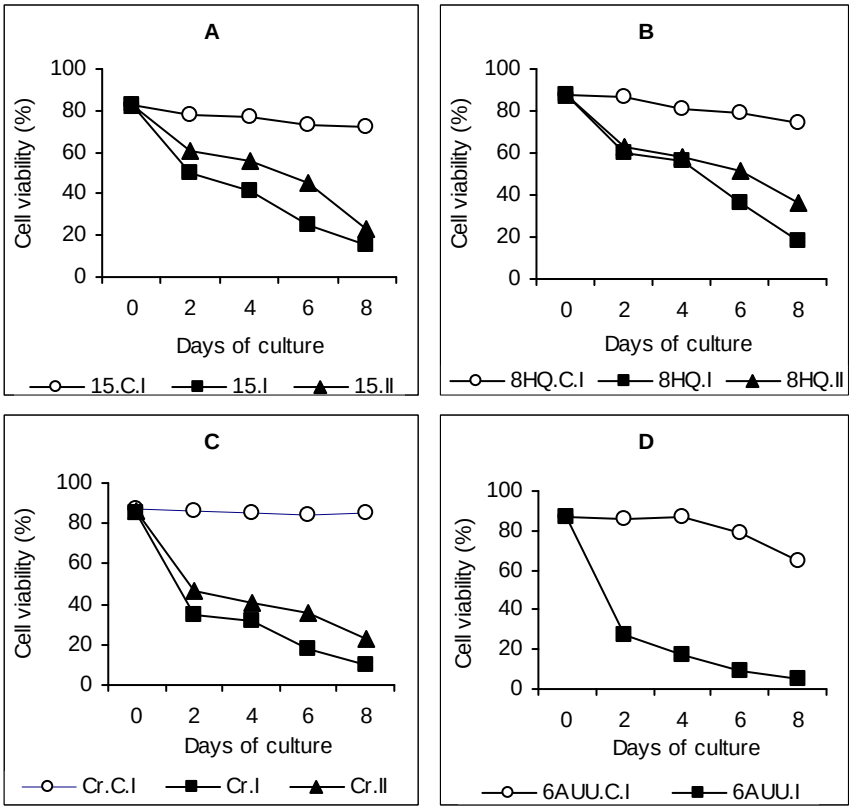

Fig. 3: Effect of HQ (660 mg/l) on cell viability of cell suspensions. A. *Digitalis lanata* (15 cell line); B. *Digitalis lanata* (8HQ cell line); C. *Catharanthus roseus* (Cr cell line); D. *Arctostaphylos uva-ursi* (6AUU cell line). C = control. Fresh weight of inoculum: I = 133 g/l; II = 233.3 g/l

Higher cell density variants resisted well to HQ action until the third day, after that cell viability decreased seriously followed of course by a decrease in arbutin production.

The most resistant variants were 8HQ (Fig. 3B), and 15 (Fig. 3A), *Digitalis lanata* cell lines, with 233g/l fresh weight. The viability of cultured cells in these variants, after 2 days HQ was added, was 63% for 8HQ cell line and 61% for 15 cell line. This is due to their morphological traits: large cell aggregates protecting inner cells against the harmful effect of HQ.

Catharanthus roseus and *A. uva-ursi* cell suspensions consisting mostly of free, small, fragile cells were more affected and after the 4'th day the majority of cells were dead (Fig. 3C and 3D).

The toxic effect of HQ is proved also when comparing variants with the control. For all controls viability increased in tandem with cell growth until 2'nd day. After that, the quantity of cell biomass decreased and also did cells' viability. This happened probably because the components of the medium were in such low amount that they could not stand up to the fast

growth of cells in the absence of HQ. In this context, HQ is assumed to generate superoxide which can react with hydrogen peroxide to form even more injurious species of oxygen, such as hydroxyl radical and singlet oxygen [3,8,12]. It is known that sugars act as a hydroxyl radical scavengers. Thus, addition of 6% sucrose in biotransformation medium showed to improve cell viability in *Catahranthus roseus* cell culture [24].

By comparing the four experimented cell lines, we found out that the highest resistance to HQ toxicity was revealed by *Digitalis lanata* cell lines (15 and 8HQ) (Fig. 4).

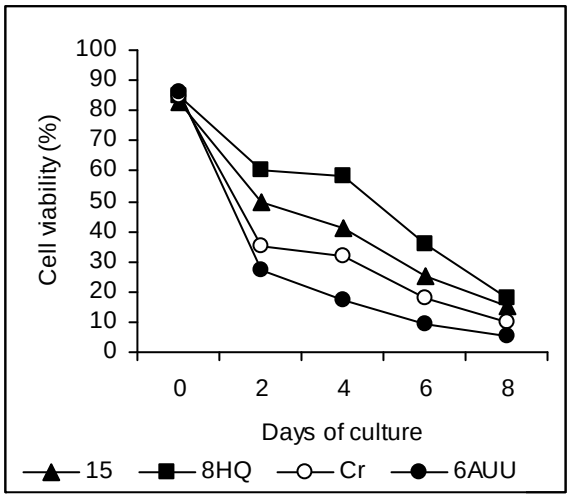

Fig. 4: Resistance of the cell suspensions to HQ (660 mg/l) action. 15 and 8HQ = *Digitalis lanata* cell lines; Cr. = *Catharanthus roseus* cell line; 6AUU = *Arctostaphylos uva-ursi* cell line. Fresh weight of inoculum =133 g/l

This resistance, as we previously pointed out, may be due both to their morphological traits and their feasibility to efficiently glucosylate other substrates too (e.g.: digitoxin) [4]. Accordingly, although expected to prove a higher resistance to the harmful effect of HQ, *A. uva-ursi* cell line demonstrated to be the most sensitive. Thus, after two days of contact with the exogenously added HQ, its viability dropped over 68% (Fig. 3D). Probable that naturally HQ is synthesized in cytoplasma of the leaf cells of this plant, but in lower amounts. These small quantities of HQ may be very fast glucosylated to arbutin, nontoxic compound, which is afterwards deposited in vacuoles. This assumption may lead to the conclusion that adding the entire dose of HQ from the beginning was not a wise choice because the concentration (6mM) we used for the substrate (HQ) was too high and therefore harmful to cultured cells.

Izoperoxidase activity in presence of hydroquinone.

Peroxidases, a large family of izoenzymes, are involved in a great number of processes such as pathogen defense, wound healing [6], auxin oxidation and lignification. The peroxidazic activity was analyzed mainly for those variants having 133.3g/l amount of the inoculum. Only for *C. roseus* cell suspension this activity was also analyzed at 233.3g/l biomass density (Fig. 5).

IEF showed various profiles of peroxidase activity, depending on the isoelectric point of the izoenzymes. Thus, at the isoelectric point of 8.5 a basic peroxidase was revealed in 2, 5 and 7, 11,12 samples of *C. roseus* and *D. lanata* 15 cell line. This peroxidase was not expressed in the corresponding controls which may conclude to the fact that this enzyme is a stress marker induced in the presence of HQ. The enzyme'activity was not detected in 8HQ *D. lanata* cell line (in the variants treated with HQ- samples: 8, 9, 10) eighter. Thus, we may assume that this cell line has a higher resistance to the toxic effect of HQ than the others, a fact which had been also proven by the cell viability. It is also possible for this cell line to own a more complex and efficient enzymatic equipment for the HQ bioconversion to arbutin, which may reduce the harmful effect of the substrate and therefore not expressing stress markers.

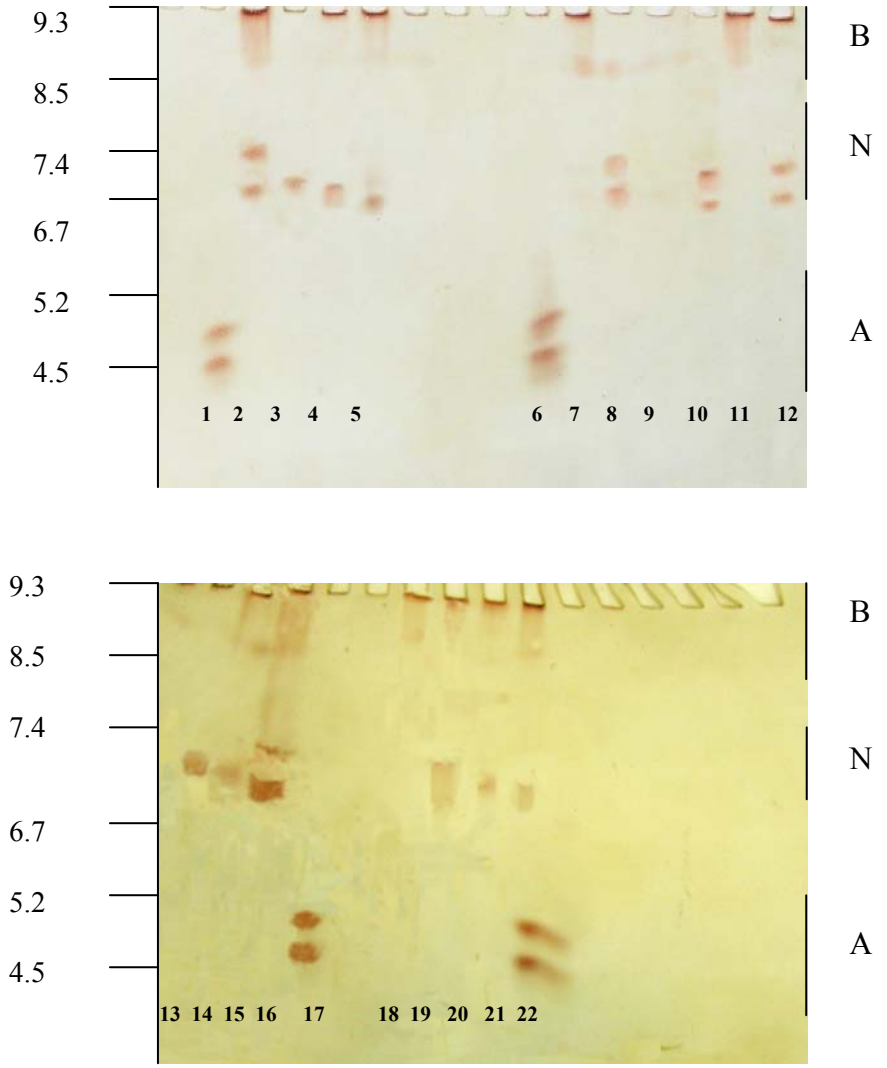

Fig. 5: Isoelectric focusing of peroxidases from cultured plant cells of *Arctostaphylos uva-ursi*, *Catharanthus roseus* and *Digitalis lanata*. pH in the gel is given on the left. Letters on the right refer to the various peroxidases of A (acidic), N (neutral), B (basic) groups. (The cell lines and variants are reproduced in Table 1).

This phenomenon was also encountered in both *Arctostaphylos uva-ursi* (with 133 g/l f.w.) and *Catharanthus roseus* (with 233.3 g/l f.w) cell lines. In the first case a possible explanation may be that considering the lower amount of the inoculum cell viabilty dropped very fast and therefore an enzymatic activity could not be detected. As for *C. roseus* cell line (samples: 3,4) with 233.3 g/l fresh weight, we can say that in correlation with the large amount of the inoculum the biotransformation process was so fast that cultured cells could not experience so intensely the harmfull effect of HQ.

Two bands were located in the neutral-slightly acid region (between 7.4-6.7 isoelectric points). These two neutral isoenzymes are expressed in all variants excepting controls, especially from the 4'th day untill the 8'th day of culture and are strongly correlated with the ageing process [11].

In the acidic region (between 5.2-4.5 isoelectric points) IEF revealed three isoperoxidases, which were expressed differently depending on the: plant species, cell lines or strains, amount of the inoculum and date of sample harvesting. These acidic forms are correlated

with the growth process being involved in biosynthesis of cell wall including lignification and suberization of the cell wall [6,11].

Table 1: The variants used for detected of izoperoxidase activity in presence of hydroquinone

Plant	Cell line	Variant	Fresh weight of inoculum (g/l)		Samples harvesting after treatment with HQ (day)			
			133	233.3	2'nd	4'th	6'th	8'th
Catharanthus rosesus	Cr	**1 (C)**	**X**		**x**			
		2	**X**		**x**			
		3	**X**			**x**		
		4	**X**					**x**
		18	**X**	**x**	**x**			
		19		**x**		**x**		
		20		**x**			**x**	
		21		**x**				**x**
		22 (C)		**x**	**x**			
Digitalis lanata	8HQ	**6 (C)**	**X**		**x**			
		8	**X**			**x**		
		9	**X**				**x**	
		10	**X**		**x**			
	15	**7**	**X**		**x**			
		11	**X**				**x**	
		12	**X**			**x**		
Arctostaphylos uva-ursi	6AUU	**13**	**X**		**x**			
		14	**X**			**x**		
		15	**X**				**x**	
		16	**X**					**x**
		17 (C)	**X**		**x**			

C = control

Hydroquinone biotransformation.

For HQ bioconversion to arbutin the same plant cell cultures were used: 6AUU *A. uva-ursi* cell line (sythesizing arbutin and hidroquinone under natural conditions), CR *C. roseus* cell line as well as 15 and 8HQ *Digitalis lanata* cell lines (species which naturally produce neigther of these two compounds). HQ was added to give a final concentration of 6 mM to suspensions of cells on the first day. The samples were collected after 2, 4, 6 and 8 days of culture. Our attention was also focused on the influence of the amount of fresh biomass on the efficiency of biotransformation process. Consequently two different amount of inoculum were used: I=133g/l f.w. and II=233g/l f.w.

Tthin layer chromathography (TLC) revealed some important aspects (Fig. 6). It is obvious that *A. uva-ursi* cell line (f.w.=133g/l) lacks completely the capacity to biotransform exogenously added HQ. One possible explanation of this phenomenon may be the fact that the concentration of HQ (6mM) is very harmfull for this suspension and most of the cells are already dead, being incapable of further activities (which is why this species is not even represented on the chromathograms). This finding is sustained by the results of cell viability as well (Fig. 3D).

Analysing the intensity of the spots on the chromathograms we concluded that the efficiency of the biotransformation process for the rest of the suspensions depended on the: plant species, cell line or strain, harvesting date and cell density. Thus, whenever harvested, for the variants with a higher cell density (f.w. = 233g/l) the intensities of the spots corresponding to arbutin (A) were significatly prominent. This leads to the conclusion that at this amount of the inoculum all suspensions proved an increased bioconversion capacity of exogenously added HQ.

The bioconversion capacity is also influenced by the time spend by cultured cells in contact with HQ. Consequently we observed that biotransformation of the substrate to arbutin goes on untill the 6'th day in the case of 15 *D. lanata* cell line only, and for the rest untill the 4'th day of culture (Fig. 6).

The selection of high production cell lines appears to be a useful means of improving the production of compounds by biotransformation in plant suspensions [7,23]. At 2 days of culture, depending on cell lines, the best bioconversion capacity was registered at: *Catharanthus roseus*

cell line and 8HQ *D. lanata* cell line. Afterwards, the accumulation of arbutin in cultured cells dropped gradually.

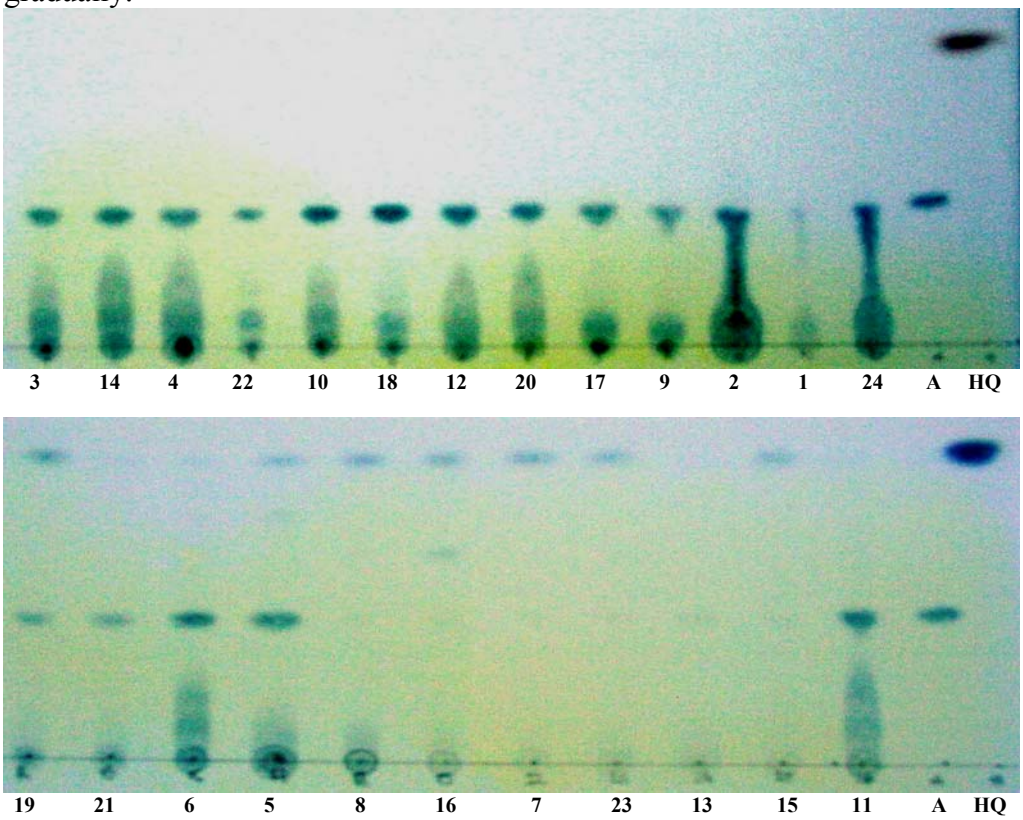

Fig. 6: Thin layer chromatography of arbutin as a result of hydroquinone biotransformation by plant cell cutures. A = arbutin; HQ = hydroquinone. The cell lines and variants are reproduced in Table 2.

Table 2: The variants used for biotransformation of hydroquinone (HQ)

Plant	Cell line	Variant	Fresh weight of inoculum (g/l)		Samples harvesting after treatment with HQ (day)			
			133	233.3	2'nd	4'th	6'th	8'th
Digitalis lanata	15	**1**	x		x			
		2		x	x			
		3	x			X		
		4		x		X		
		5	x				x	
		6		x			x	
		7	x					x
		8		x				x
	8HQ	**9**	x		x			
		10		x	x			
		11	x			X		
		12		x		X		
		13	x				x	
		14		x			x	
		15	x					x
		16		x				x
Catharanthus roseus	Cr	**17**	x		x			
		18		x	x			
		19	x			X		
		20		x		X		
		21	x				x	
		22		x			x	
		23	x					x
		24		x				x

These thorough going observations demonstrated that HQ bioconversion to arbutin depended in a large measure on: plant species, cell lines and their morphophysiological traits. In our case the best producing cell lines (CR and 8HQ) consisted of large cells equipped with well-developed vacuoles, which proved to be very advantageous for the process'efficiency.

Yamamoto et al. [22] reported that vacuoles in high-alkaloid-producing cell lines of *Coptis japonica* and *Thalictrum minus* were larger than those in low- or nonproducing cell lines. They suggested that development of vacuoles may be a prerequisite for alkaloid synthesis, to prepare a reservoir for storage of the alkaloids. In the case of *Catharanthus roseus* B cell line, Yokoyama and Inomata [24] observed that the cells expanded about two fold with a corresponding expansion of the vacuoles as the accumulation of arbutin progressed and the bioconversion was higher than in the case of *Catharanthus roseus* A cell line consisting of smaller, globular cells.

Conclusions

The results of the biotransformation of HQ to arbutin by *Arctostaphylos uva-ursi*, *Catharanthus roseus* and *Digitalis lanata* cell cultures may be summarized as follows:

1. Selection of a superior cell strain was important for the efficient production of arbutin (e.g.: 8HQ a hormone habituated cell line with induced tolerance to HQ, with well developed vacuoles and large cell aggregates).
2. Cell growth rate is undoubtedly higher in the absence of HQ (for controls) and also in the case of lower cell density variants because of a richer amount of some components in the medium.
3. Cell viability was higher in those variants with larger amount of biomass, proving higher tolerance to HQ and therefore biotransforming better this substrate.
4. A basic isoperoxidase involved in stress response has been expressed differencially as a result of HQ addition to the culture media.
5. Neutral and acidic peroxidases were expressed differently depending on cell lines and duration of contact with HQ.
6. Higher cell density variants possessed higher ability to produce arbutin than lower cell density variants (CR and 8HQ cell lines)**.**
7. Accumulation of arbutin was observed generally in cells.
8. The biosynthetic potential of some species is very often not expressed in cell culture, thus *Arctostaphylos uva-ursi* cell line proved the lowest capacity to biotransform HQ to arbutin.

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ASPECTE PRIVIND EFECTUL HIDROCHINONEI ASUPRA CULTURILOR CELULARE DE *ARCTOSTAPHYLOS UVA-URSI, CATHARANTHUS ROSEUS* ŞI *DIGITALIS LANATA*

(Rezumat)

Culturile celulare vegetale biotransformă o gamă largă de substrate, aşa cum este şi hidrochinona (HQ), compus toxic. În urma biotransformării, HQ poate fi detoxificată, prin glucozilare, rezultând arbutina. Pentru biotransformarea HQ s-au utilizat culturi celulare de *Arctostaphylos uva-ursi* şi *Catharanthus roseus*, precum şi două linii celulare de *Digitalis lanata* (15 şi 8HQ). Introducerea HQ în mediul lor de cultură, în concentraţia de 660 mg/l, a avut efecte diferite asupra unor procese fiziologice (creştere, viabilitate şi activitate peroxidazică). Intensitatea acestora a fost influenţată de mai mulţi factori: de specie sau de linia celulară, de cantitatea de biomasă celulară utilizată ca inocul şi de durata de contact a celulelor cu HQ. Astfel, deşi creşterea celulară a fost aparent mai puţin afectată, viabilitatea celulară a fost puternic inhibată în toate culturile celulare. Cele mai rezistente la acţiunea toxică a HQ s-au dovedit a fi liniile celulare 15 şi 8HQ de *Digitalis lanata*, iar cea mai sensibilă, cultura celulară de *Arctostaphylos uva-ursi*. În funcţie de gradientul de pH, s-a evidenţiat o izoperoxidază bazică, considerată a fi o

peroxidază de stres, care s-a exprimat în culturile celulare de *Catharanthus roseus* şi *Digitalis lanata* (linia 8HQ). Absența acestei izoperoxidaze la linia celulară 8HQ de *D. lanata* poate explica rezistența celulelor la acțiunea toxică a HQ. Celelalte izoperoxidaze identificate sunt asociate procesului de creştere celulară, exprimarea lor fiind diferențiată în funcție de specie, de linia celulară şi de cantitatea de inocul. În ceea ce priveşte capacitatea de glucozilare a HQ, analizele calitative (TLC) au scos în evidență, faptul că, cu excepția celulelor de *Arctostaphylos uva-ursi*, toate celelalte culturi celulare biotransformă HQ în arbutină. Cele mai eficiente culturi celulare, din acest punct de vedere, au fost cele de *Catharanthus roseus* şi de *Digitalis lanata* (linia celulară 8HQ), la două zile după introducerea HQ în mediul de cultură.