Colchicine-induced resistance to antibiotic and amino-acid analogue in plant cell cultures

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Summary. Colchicine-resistant plant cell strains have been isolated from cell suspensions of carrot and sycamore. In the same way as colchicine-resistant animal cell strains, they show an increased resistance towards streptomycin and N-methyl-alanine. Cultivation under non selective conditions leads to a slow progressive loss of the resistance.

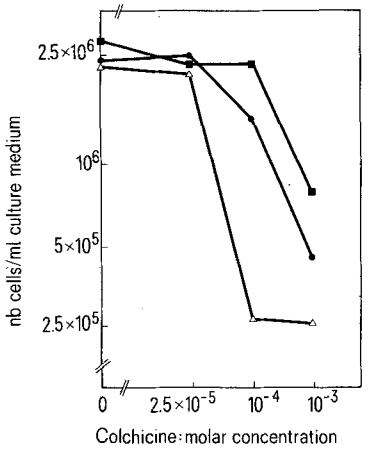
In the course of a systematic study designed to test the sensitivity of cultured plant cells to various drugs, it was found that colchicine-resistant cell strains can be isolated from sycamore (*Acer pseudoplatanus* L.) and carrot (*Daucus carrota* L.) cell suspensions, after plating on selective media. Colchicine-induced resistance to other antimetabolites has been demonstrated in animal cell cultures, where it is linked to changes in plasmalemma permeability[1,2].

Cell suspensions of sycamore and of carrot were cultivated respectively in an artificially conditioned medium[3] and in MS medium[4]. Both media contained 1 mg 2,4-dichlorophenoxyacetic acid/l, their pH was adjusted to 5.6. Cells from exponentially growing cultures were plated at a density of 10^3 germs/ml (germs are the small cell aggregates remaining after filtration through a 150 µm opening nylon filter) on their respective media solidified with 0.6% agar. To avoid phototransformation of colchicine, all experiments were performed under dim green light or complete darkness[5].

Plant cells are fairly resistant to colchicine[6], and up to 10^{-4} M, no significant growth inhibition occurs. Therefore we have used concentrations up to 2.5 10^{-3} M in the selection process. The drug was filter-sterilized before use. After some weeks of incubation in darkness, some colonies started to develop at concentration of $2.5 \cdot 10^{-4}$ M or even higher, depending of the species (table 1). Attempts to increase the frequency of colony formation at high colchicine concentrations by mutagenic treatments (with ethylmethyl sulfonate or N-nitroso-N-methylguanidine) have so far been unsuccesful. Well developed colonies were picked up from the highest drug concentration experiment and subcultured in presence of the same concentration of colchicine; after 6 weeks they were transfered to a nonselective medium and tested for the stability of the resistant character. After 1 or 5 21-day passages, cells were tested for their susceptibility to various colchicine concentrations (figure). A progressive increase in growth inhibition with the number of passages can be demonstrated. After 1 year, the CR 5 line of sycamore cells (colchicine resistant) was no longer able to grow in presence of 10^{-4} M colchicine. This suggest that, either isolated colonies were nonhomogenous with respect to colchicine resistance and therefore under nonselective conditions resistant cells were slowly selected out, or colchicine resistance is an unstable property due to a transient alteration of gene expression[7,8].

The morphology of the resistant strains of both carrot and sycamore is different from that of the main strains: cells were colourless (giving a white appearance to the colonies) and were spread on the surface of agar in a soft mass of isolated cells and small groups of less than 4 cells. These morphological features persist with the resistance to high colchicine levels; they are very similar to those of soja-bean cells grown in presence of 10^{-4} M colchicine[9].

When resistant cells are plated in a medium containing other antimetabolites, they show an increased resistance to those substances. Demonstration of this fact can be made by testing streptomycine, a specific inhibitor of plastid



Transient resistance to colchicine in sycamore CR strain. Control, N strain (-△-) and resistant CR strain cultivated for 1 (-■-) or 5 passages (-●-) on drug-free medium, are plotted. Each point is the average of triplicate cultures.

Table 1. Selection of colchicine resistant strains: plating efficiency at increasing colchicine concentrations

Colchicine concentrations	0	$2.5 \cdot 10^{-5}$M	$2.5 \cdot 10^{-4}$M	$2.5 \cdot 10^{-3}$M
Carrot cells	35	2.5	0.001	0
Sycamore cells	18[a]	11[a]	0.03	0.0005

Plating efficiency is expressed as percent of germs developed as colonies (>0.35 mm diameter) after 3–8 weeks of incubation (28 °C, in darkness). Data shown are the pooled results of 4 different experiments. Results in absence of colchicine can only be considered as approximations, due to overlap of developed colonies. Results labeled by the same letter cannot be considered as significantly different (at 95% significance level).

Table 2. Comparison between the plating efficiences of the normal (N) and colchicine resistant strains (CR 5) of sycamore cells: sensitivity towards various concentrations of 2 antimetabolites: streptomycine and N-methyl-alanine

Strains	Drug concentrations (mg/ml) 0		0.1		1		10	
	N	CR5	N	CR5	N	CR5	N	CR5
Streptomycine	26[a]	17[a]	0	2.7	0	0.05	0	0
N-methyl-alanine	15[b]	21[b]	0.2	5.2	0	0.4	0	0.007

Plating conditions are the same as in table 1, as well as the presentation of results.

protein synthesis[10] and N-methyl-alanine, an analogue of alanine which interferes with a large number of biosynthetic pathways[11]. A 10- to 100-fold increase of the resistance towards both substances can be observed (table 2). Suggestions have been made that, in animal cell, such nonspecific larger resistance can be ascribed to changes in membrane permeability[1,2]. This phenomenon is, as we have shown, not restricted to animal cells, but extends also to plant cells. Plant cells contain only a small specific binding activity towards colchicine, and the highest resistance of plant cells has been ascribed to a nonspecific binding component present at the cell surface or in the cell wall[5,12]. In this respect, the observed loosening of the cell aggregates in the resistant cell strains may be due to modifications of the cell wall components and will be correlated with an increase of the nonspecific binding. The observations made by some authors that cytoplasmic microtubules associated with cellulose microfibrils deposition are disrupted by colchicine[13], and that some binding activity is associated with the membrane fraction in plant cells[14] may also lead to the conclusion that resistance is closely associated with modifications of the plasmalemma. The resistance spectrum acquired by colchicine-resistant plant cells can therefore be linked to changes in plasma membrane properties, leading to a decrease in permeability.

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Histochemical evidence on the accumulation of sulfated proteins in the innermost laticifers of the *Euphorbia marginata* embryo

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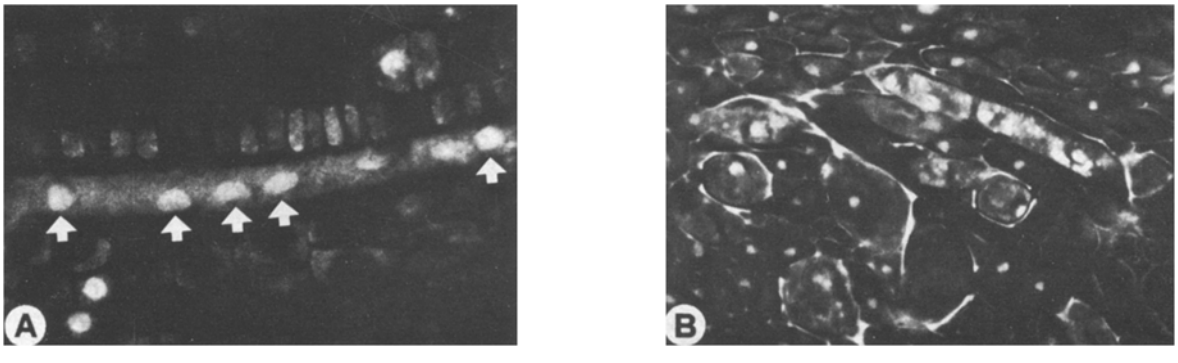
Summary. The presence of proteins containing sulfhydryl and disulfide groups was demonstrated by fluorescent mercurials in the cytoplasm of the innermost embryonal laticifers of *Euphorbia marginata*. The finding is discussed in the context of the role of the embryonal laticifers.

The mature embryo of *Euphorbia marginata* displays a complex latex system morphologically recognizable in vascular, cortical and cotyledonar components[1,2]. During germination a progressively degenerating cytoplasm occurs in the laticifers, and the changing in its content is the first symptom of the lytic-machinery activation for the latex synthesis[3-5]. In this work, some methods normally employed in the detection of animal protein-bound sulfhydryl and disulfide groups[6], have been optimized to evaluate the content in sulfur-containing proteins of the embryonal laticifers, and, consequently, to furnish some indications on the differentiation pattern of these internal secretory structures.

Embryos of *Euphorbia marginata* were fixed in absolute ethanol-acetic acid (3:1), embedded in a butyl-methyl methacrylate mixture and sectioned at 1 µm by a microtome with glass knives. The sections were placed on slides and the plastic medium was removed by a xilene-benzene mixture. To detect SH groups, mercury orange (20 mg dye/100 ml N,N-dimethylformamide) or mercurochrome (20 mg dye/100 ml water) were used. The preparations were placed in the dye solution for 30 min, rinsed in the dye solvent, passed through absolute ethanol, and cleared in xilene. Coverslips were mounted with a free fluorescence medium. The sum of SH and SS groups was demonstrated after reduction by thioglycolic acid[7], and the blocking reactions were made with iodoacetate or N-ethyl maleimide[8]. The optical system used consisted of a Zeiss Photomicroscope II equipped with a HBO 200 W lamp, a reflecting condenser, a 460 nm dichroic mirror, and a series of barrier filters.

When sections were examined by light fluorescence micros-



Innermost laticifers in *Euphorbia marginata* embryo strongly fluorescent by organomercurials: *A* The cytoplasm of a vascular laticifer is positive in the localization of SH plus SS groups (the arrows indicate the nuclei). ×480. *B* Fluorescent laticifers of the latex plexus indicating the presence of SH plus SS groups. ×380.