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ISOLATION OF CELL LINES WITH DECREASED OR DEFICIENT NITRATE-REDUCTASE ACTIVITY FROM CELL-SUSPENSION CULTURE OF MONO-($2n=x=12$) AND DIHAPLOID ($2n=2x=24$) *SOLANUM TUBEROSUM* PLANTS[1]

BOŻENA CHWIŁKOWSKA[2]

Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland

Summary. In order to obtain in vitro cell lines resistant to high chlorate concentrations, suspension-incubated cells of mono-($2n=x=12$) and dihaploid ($2n=2x=24$) *Solanum tuberosum* plants were exposed to the action of gamma-rays (500 r/min.) at the doses of 4 and 5 kR. These doses had an inhibiting effect on the plating efficiency and cell survival in the control medium. A small per cent of nonradiated cells also survived in the selection medium containing chlorates in the cultures of dihaploid ($2n=2x=24$) cells. An increase in the plating efficiency of cells radiated in the selection medium may indicate induced mutagenesis consisting in the loss of nitrate reductase activity in these cells. Ten chlorate-resistant cell lines, selected from radiated cells, successfully developed for 3 years on the selection medium with a high chlorate concentration and when passaged on the control medium for over a year, have not restored the properties of wild type cells, thus becoming „stable variants" according to Müller, Grafe (1978). The selected cell lines differ by such morphological characters, as the colour, structure, the mode and intensity of growth of the callus tissue on the following media: control, selective and nitrate medium containing 3920 mg/l KNO_3. Coefficients of the callus growth of chlorate-resistant cell lines on the nitrate medium range from 0.89 to 4.20 and are lower than those of the control lines (6.08 - 6.71). This indicates a lowered NR activity or its lack in the selected cell lines.

Development of in vitro culture techniques, such as: the obtaining of haploid plants; protoplast isolation, culture and fusion; single cell culture in the suspension; mutagenesis of in vitro protoplasts or cell—suspension culture and their clonal selection for various selection factors followed by their application in combination with genetic engineering techniques—makes possible pursuance of research in the field of somatic cell genetics in higher plants.

Somatic cell genetics of higher plants develops in the theoretical aspect, on the one hand, and concerns studies of mechanisms of genetic control and regulation of various metabolic cycles on the cellular level and in the practical aspect, on the other hand, to provide new forms of economically important plants. The method of in vitro cell mutagenesis and then, clonal selection of variation products to dif-

[1] Received for publication: January 1988.

[2] Dr. Present address: Strzeszyńska 34, 60-479 Poznań, Poland.

ferent selection factors, open large research perspectives in the field of somatic cell genetics. That method permitted to obtain cell lines or regenerated plants resistant to some antibiotics, herbicides, bacterial and fungal toxins, as well as cells characterized by overproduction of exogenic amino acids.

An important field of experimental genetics studies on the cellular level is a study of mechanisms of genetic control and regulation of mineral nitrogen assimilation in higher plants. These studies are conducted on the basis of cell mutations consisting in differentiated abilities of assimiliation of various nitrogen forms. The method of mutagenesis of in vitro cells-suspension culture and their clonal selection for high chlorate concentrations permits the obtaining of cells and tissues with genetic changes of enzymes participating in mineral nitrogen assimilation, including cell lines characterizing by an decreased nitrate reductase (NR) activity or by its lack (NR^-).

The system of cell selection for chlorates lies in that active nitrate reductase (NR) in cells cultured on $KClO_3$ selection medium converts $KClO_3$ into $KClO_2$, toxic to cells. In this connection only cell-mutants with inactive nitrate reductase can survive on account of their physiological resistance to chlorates and nitrate reductase deficiency. The selection method employed was based on the observation that the toxicity of chlorate to plant cells depends on NR activity, presumably because NR catalyses the reduction of chlorate to highly toxin chlorite (Aberg 1947). To avoid competition between nitrate and chlorate, nitrate-free selection media containing amino acids or ammonium succinate as the only nitrogen source were used.

The above selection system was used in studying mechanisms of genetic control and regulation of mineral nitrogen assimilation in bacteria (Puig et al. 1967), fungi (Cove 1976; Singh et al. 1977), algae (Sosa et al. 1978), which resulted in the obtaining, for the above purposes, of cells with genetic changes of enzymes participating in mineral nitrogen assimilation including nitrate reductase deficient cells of the type nia and cnx.

On the basis of mutagenesis of in vitro cells-suspension culture and their clonal selection for high chlorate concentrations, cell and plant characterizing by genetic changes of enzymes participating in mineral nitrogen assimilation were obtained in higher plans — in many species, such as: *Nicotiana tabacum* (Müller, Grafe 1975, 1978, Mendel, Müller 1979, 1980, Mendel et al. 1981, 1982, Mendel 1983, Mendel et al. 1984), *Pisum sativum* (Feenstra, Jacobsen 1980, Warner et al. 1982), *Hordeum* (Kleinhofs et al. 1980, 1983), *Nicotiana plumbaginifolia* (Marton et al. 1982), *Datura innxia* (King, Khanna 1980), *Hyoscyamus muticus* (Strauss et al. 1981), *Arabidopsis thaliana* (Braaksma, Feenstra 1982), *Petunia* (Steffen, Schieder 1983). Such mutants, and particularly cells and plants with nitrate reductase deficiency (NR^-), can be used in the studies of genetic control and regulation of mineral nitrogen assimilation in higher plants (Mendel, Müller 1976, Müller, Grafe 1978, Mendel, Müller 1979, Kleinhofs et al. 1980, 1983, King, Khanna 1980, Mendel et al. 1981, Strauss et al. 1981, Braaksma, Feenstra 1982, Buchanan, Wray 1982, Marton et al. 1982, Warner et al.

1982, Müller 1983, Somers et al. 1983, Steffen, Schieder 1983, Mendel, Müller 1985). Besides that, mutants of this type are supplied with an effective selection system making possible the obtaining of somatic hybrids and cybrids (Müller, Grafe 1978, Glimelius et al. 1978, Evola et al. 1983, Lazar et al. 1983, Hamill et al. 1984, Pental et al. 1984, Negrutiu et al.1986,) which are used in the studies of complementary traits. This system is characterized by that cells and plants with nitrate reductase—deficiency (NR^-) are not capable of growing and developing on the medium, in which the only source of nitrogen are nitrates. But they survive in the presence of ammonium or amino acids. Ammonium salts can be assimilated on condition that tricarboxylic acid cycle (TCAC) indirectly participates in it (Müller, Grafe 1978). Hamil and Cocking (1986) showed that if media used for the culture of nitrate reductase—deficient (NR^-) cells and plants are supplemented with succinate, then, ammonium assimilation by such cells and plants may increase, but on condition that pH of the media is stabilized by buffer, for instance, MES.

The purpose of the present studies was to obtain, on the basis of radiation-induced mutagenesis of suspension-incubated monohaploid ($2n=x=12$) and dihaploid ($2n=2x=24$) *Solanum tuberosum* cells followed by their clonal selection for high chlorate concentrations, cell lines characterized by genetic changes of enzymes participating in mineral nitrogen assimilation, which will serve to study genetic control and regulation of mineral nitrogen assimilation in potatoes and in the future — to obtain cells and tissues with an increased nitrogen assimilation ability.

MATERIAL AND METHODS

The studying material consisted of dihaploid ($2n=2x=24$) plants of *Solanum tuberosum* of the line Pl. 620 obtained from the variety Everest by crossing plants ($2n=4x=48$) of that variety with *Solanum phureja* ($2n=2x=24$) (Jakubiec 1964, Chwiłkowska, Zenkteler 1978) and monohaploid ($2n=x=12$) plants of *Solanum tuberosum* of the line m. 620 obtained by pollinating dihaploid ($2n=2x=24$) plants of the line Pl. 620 with pollen of *Solanum tarijense* ($2n=2x=24$) (Chwiłkowska, Zenkteler 1982). Monohaploid and dihaploid plants under conditions of in vitro cultures were obtained by transferring the meristems of lateral and apical buds onto the A medium containing macro- and microelements according to Murashige and Skoog (1962), thiamine — 1.0 mg/l, casein hydrolysate — 500 mg/l, mesoinositol — 100 mg/l, sucrose — 30 g/l, Difco-Bacto agar — 7 g/l and growth substances: kinetin — 1.0 mg/l, IAA — 0.5 mg/l. Plant obtained after a 6-week incubation of the meristems were vegetatively propagated. Shoot tips as well as shoot fragments (nodes) with lateral buds were transferred onto a fresh A medium. The plants were incubated in tubes in light with the intensity of 2000 lx at a 14-h photoperiod and a temperature from 23°C to 25°C (Khvilkovskaya (— Chwiłkowska) 1982).

The callus tissues were obtained as a result of culture of shoot explants of mono- and dihaploid plants on C agarose medium containing macro- and microelements

according to MS (1962), thiamine — 1.0 mg/l, mesoinsoitol — 100 mg/l, casein hydrolysate — 500 mg/l; growth substances: 2.4 D — 2.0 mg/l, kinetin — 0.2 mg/l, NAA — 0.2 mg/l; sucrose — 30 g/l, Difeo-Bacto agar — 7.0 g/l, pH 5.7 (Khilkovskaya (— Chwiłkowska) 1982). Shoot explants were incubated in the dark at the temperature of 26°C.

Cell cultures in the suspension were obtained from callus tissues, which were mechanically crushed and incubated in a liquid medium C (Khvilkovskaya (— Chwiłkowska) 1982). Cell of stabilized suspension cultures of the 3rd passage, being at the stage of logarithmic growth (14th day for m. 620 cells and 18th day for P1. 620 characterized by the highest PE) for m. 620 cells — 40.85% and P1. 620 — 68.0% (— Chwiłkowska (in preparation)) in the volume of 40 ml with the density of 4×10^4 cells/cm^3, were exposed to the action of gamma-rays (500 r/min.) i. e. irradiated at the doses of 4 and 5 kR (these are average doses among the applied ones — 3, 4, 5, and 6 kR, they were chosen as optimal, since the dose of 3 kR stimulated cell divisions in relation to the control, whereas the dose of 6 kR was lethal for the above cells) for 8 and 10 min. After washing radiated cells by a liquid medium C and after 1.5-fold dilution, they were incubated in the suspension for 24 hours. Then, radiated as well as nonradiated cells were plated in agar media: control (CM) with the aim to trace the influence of gamma rays on plating efficiency (PE) and cell survival (S), and selection (SM-I) with the aim to select mutated cells resistant to chlorates.

Control medium contained macro- and microelements according to MS (1962), thiamine — 1.0 mg/l, mesoinositol — 100 mg/l, casein hydrolysate — 500 mg/l; growth substances: 2.4 D — 2.0 mg/l, kinetin — 0.2 mg/l, NAA — 0.2 mg/l; sucrose — 30 g/l; Difco—Bacto agar — 8.0 g/l, pH 5.7.

Selection medium contained mineral nitrogen only in the form of NH_4NO_3 — 1.0 g/l; the remaining salts entering into the composition of macro- and microelements according to MS (1962); vitamins, growth substances, sucrose, agar as in the CM medium; organic nitrogen in the form of casein hydrolysate — 2.0 g/l and selection factor $KClO_3$ — 2.5 g/l (Müller, Grafe 1975; Levenko 1981), which in the case of the presence of active nitrate reductase (NR) in the cell is reduced to $KClO_2$ toxic to cells. In this way, only those cells survive on the selection medium, which are incapable of reducing $KClO_3$, since they have no or have a decreased nitrate reductase activity. The plating density was $5.3 \cdot 10^3$ cells/cm^3. The cells were incubated in 5 ml agar media CM and SM-I on the spacer in Petri dishes of 8 cm diameter. The cultures were conducted for 2 - 3 months in light and in the dark with 5 replications, i. e. 5 Petri dishes for each combination.

Results of the studies concerning plating efficiency and cell survival on the media CM and SM-I of irradiated and nonirradiated cells of mono- and dihaploid *S. tuberosum* plants are presented in Table 1.

In order to test the stability of the acquired resistance of cells to high chlorate concentrations, the selected cell colonies on the selection medium SM-I were inoculated into the selection medium SM-II. The SM-II medium contrary to the SM-I medium contained no mineral nitrogen, but only organic nitrogen in the form of

Table 1. Plating efficiency, survival in control and selection media of irradiated and non-irradiated mono-($2n=x=12$) and dihaploid ($2n=2x=24$) cells of *S. tuberosum* plants incubated in light and in the dark, and frequency of chlorate resistant colonies. The plating density was 5.3×10^3 cells/cm^3

S. tuberosum cells descending from lines	Dose of gamma-rays	Plating efficiency in per cent				Survival				Frequency of chlorate-resistant colonies	
		control medium		selection medium		control medium		selection medium		selection medium	
		dark	light	dark	light	dark	light	dark	light	dark	light
Pl. 620 ($2n=2x=24$)	0 kR	67.06	65.71	12.13	9.68	1.00	1.00	0.18	0.15	0.12	0.09
	4 kR	46.53	45.71	28.94	19.26	0.69	0.69	0.43	0.29	0.28	0.19
	5 kR	36.94	38.17	22.15	15.77	0.55	0.58	0.33	0.24	0.22	0.15
m. 620 ($2n=x=12$)	0 kR	30.62	31.85	0.00	0.00	1.00	1.00	0.00	0.00	0.00	0.00
	4 kR	15.69	16.07	10.73	7.24	0.51	0.50	0.35	0.22	0.10	0.07
	5 kR	11.36	11.96	7.66	5.87	0.36	0.37	0.25	0.18	0.07	0.05

casein hydrolysate — 2.0 g/l; microelements and the remaining salts of macroelements according to MS (1962); vitamins, growth substances, sucrose (Khvilkovskaya (— Chwiłkowska) 1982), agar as in the CM medium and $KClO_3$ — 2.5 g/l (Müller, Grafe 1875, Levenko 1981), which selected cells with active NR. Only those chlorate-resistant cells survived on the SM-II medium, which could assimilate organic nitrogen in the form of amino acids. Cell colonies selected on the SM-I medium were passaged onto the SM-II medium to Petri dishes 10 cm in diameter. 20 colonies were inoculated into each dish, after which they were passaged at 2-month intervals onto a fresh SM-II medium up to the 7th passage, i.e. until the time, when the number of inoculated cell colonies (clones) in individual combination was 20 to 30 (Table 2.). Then each clone was cloned and placed into separate Petri dishes 6 cm diameter. Thus propagated clones, derived from individual cell colonies, placed in separate Petri dishes, simultaneously constituted separate cell lines — but that was until the time, when all cloned callus fragments of a given clone preserved stability with regard to such morphological characters, as the colour, structure and intensity of the callus growth. When some callus fragment of a given clone began to differ from the remaining ones by the mentioned morphological characters, it was isolated and cloned in a separate Petri dish 6 cm in diameter and then passaged each two month onto a fresh SM-II medium as described above, constituting thereby a new cell line derived from a given clone (descending from the cell colony formed as a result of a single cell division).

Selection of cells on the SM-II medium lasted for 3 years. The selected cell lines were preliminarily characterized regarding such morphological characters, as the colour, structure, growth and intensity of the callus tissue increase on the CM and SM-II media. Coefficients of the callus growth of the control (No. 1, No. 12) and chlorate-resistant (No. 2 - 11) cell lines on the CM, SM-II and n-SM media (after over 3-years selection) presented in Table 3 are a multiplicity of the initial weight.

Nitrate medium — (NM) contained mineral nitrogen only in the form of KNO_3 (3920 mg/l); microelements and the remaining salts of macroelements according

Table 2. The frequency of *S. tuberosum* cell line resistant variants (selected on the SM-I medium) in the subsequent passages during 3-year selection on the SM-II medium, incubated in the dark and in light

S. tuberosum cells descenting from lines	Dose of gamma-rays	Cell colonies	Selection medium SM-II passage											
			$p=1$		$p=2$		$p=5$		$p=7$		$p=10$		$p=(11-18)$	
			dark	light	dark	light	dark	light	dark	light	dark	light	dark	light
Pl. 620 ($2n=2x=24$)	0 kR	VF	0.12	0.09	4.0×10^{-2}	3.1×10^{-2}	3.6×10^{-4}	–	–	–	–	–	–	–
		n	300	300	50	47	1	–	–	–	–	–	–	–
	4 kR	VF	0.28	0.19	0.17	0.10	3.3×10^{-2}	1.9×10^{-2}	1.4×10^{-2}	7.6×10^{-3}	2.9×10^{-3}	1.5×10^{-3}	–	3.8×10^{-4}
		n	500	500	300	280	58	50	25	20	5	4	–	1
	5 kR	VF	0.22	0.15	1.7×10^{-2}	9.4×10^{-3}	2.6×10^{-2}	1.2×10^{-2}	1.3×10^{-2}	6.3×10^{-3}	2.2×10^{-3}	6.3×10^{-3}	1.7×10^{-3}	6.3×10^{-4}
		n	500	500	400	300	60	40	30	20	5	2	4	2
m. 620 ($2n=x=12$)	0 kR	VF	–	–	–	–	–	–	–	–	–	–	–	–
		n	–	–	–	–	–	–	–	–	–	–	–	–
	4 kR	VF	0.10	0.07	4.2×10^{-2}	3.1×10^{-2}	1.1×10^{-2}	5.7×10^{-3}	5.9×10^{-3}	3.8×10^{-3}	–	1.4×10^{-4}	–	1.4×10^{-4}
		n	500	500	200	220	55	40	28	27	–	1	–	1
	5 kR	VF	0.07	0.05	2.7×10^{-2}	1.7×10^{-2}	–	–	–	–	–	–	–	–
		n	300	300	110	90	–	–	–	–	–	–	–	–

VF – frequency of resistant cell colonies in individual passages on the SM-II medium

n – the number of resistant cell colonies subjected to selection on the SM-II medium

Plate 1

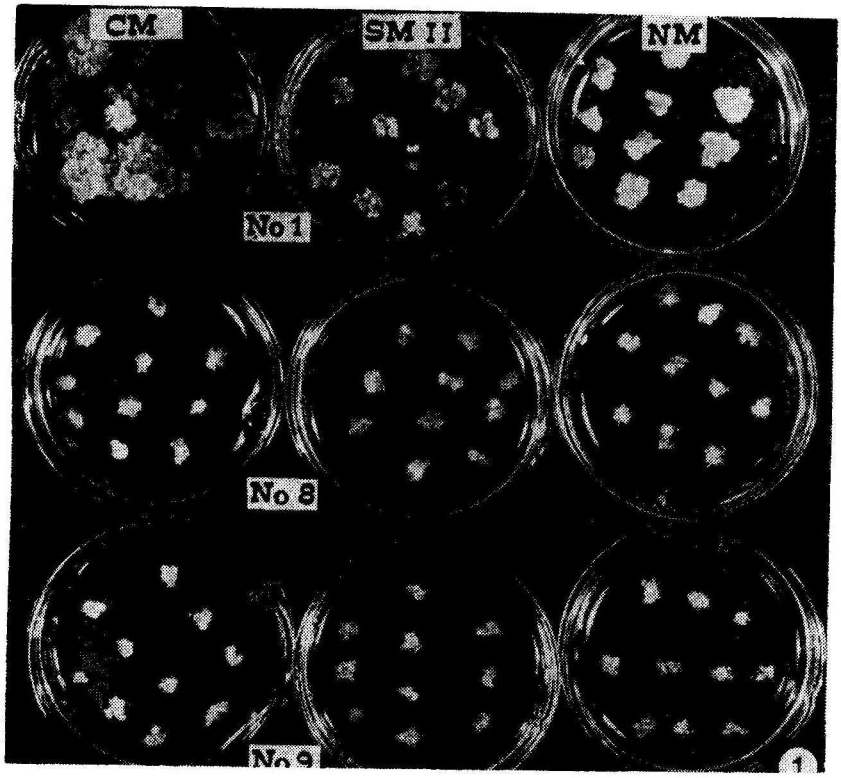

Fig. 1. Growth on the control (CM), selection (SM-II) and nitrate (NM) media of callus tissues of control line (No. 1) selected from nonradiated dihaploid *S. tuberosum* cells and chlorate-resistant cell line (No. 8 and 9) selected from dihaploid cells radiated with gamma-rays at the dose of 4 kR

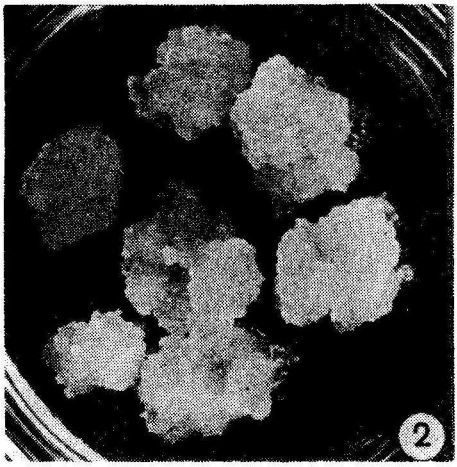

Fig. 2. Chlorate-resistant cell line No. 2 (selected from dihaploid cells radiated with gamma-rays at the dose of 5 kR) having callus tissue of a tender structure and lemon-yellow colour, on the selection medium SM-II

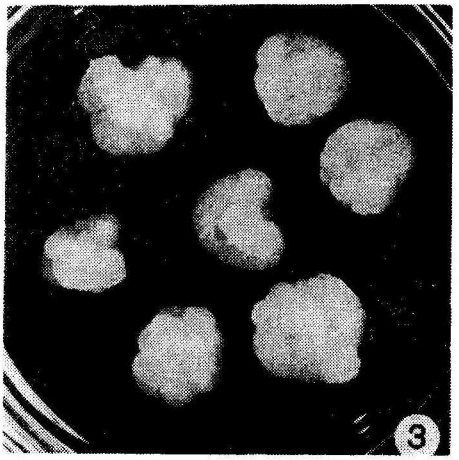

Fig. 3. Chlorate-resistant cell line No. 3 (selected from dihaploid cells radiated with gamma-rays at the dose of 5 kR) having callus tissue of a compact structure and white colour, on the selection medium SM-II

Plate 2

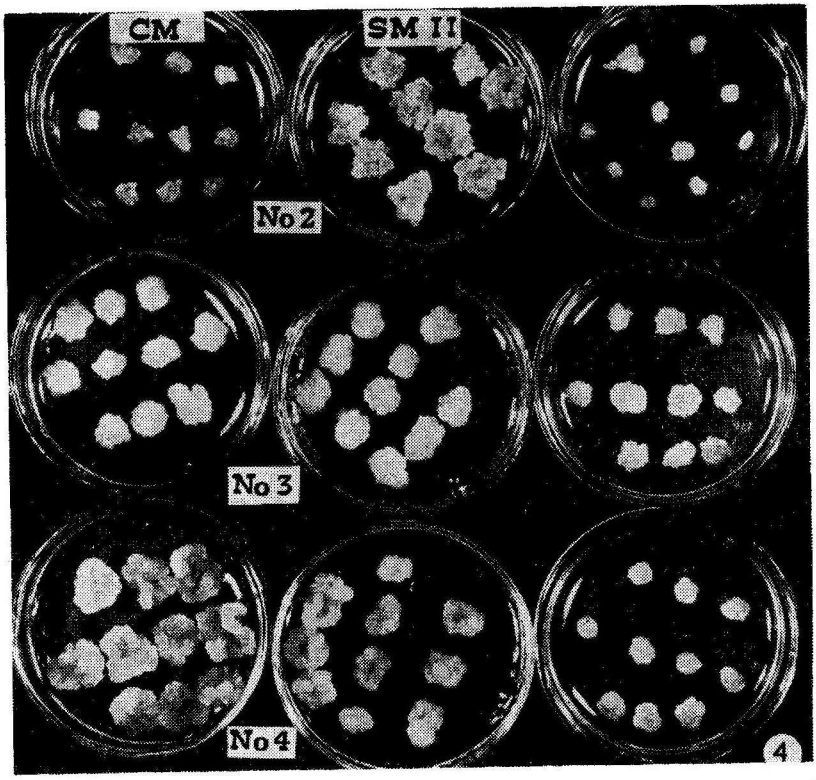

Fig. 4. Growth on the control (CM), selection (SM-II) and nitrate (NM) media of callus tissues of chlorate-resistant lines (lines No. 2 - 4) selected from dihaploid *S. tuberosum* cells radiated with gamma-rays at the dose of 5 kR

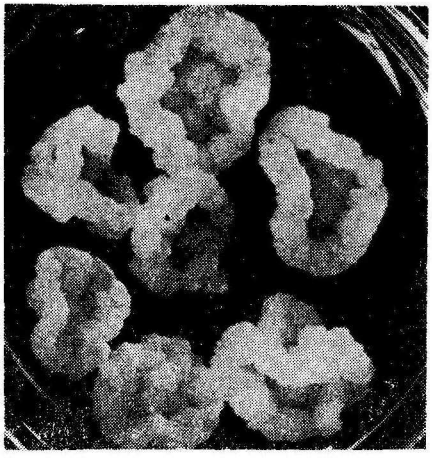

Fig. 5. Chlorate-resistant cell line No. 4 having callus tissue of a compact structure and white colour, on the selection medium SM-II

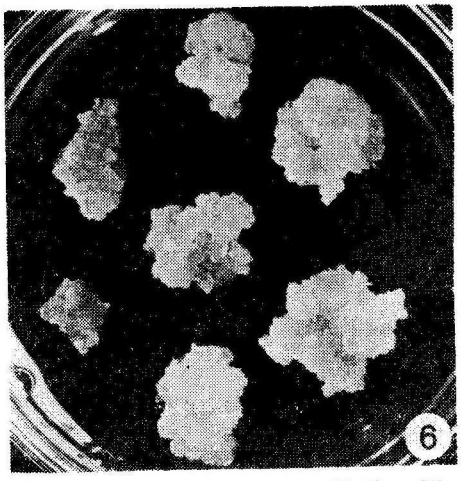

Fig. 6. Chlorate-resistant cell line No. 5 having callus tissue of a tender structure and rust colour, on the selection medium SM-II

Plate 3

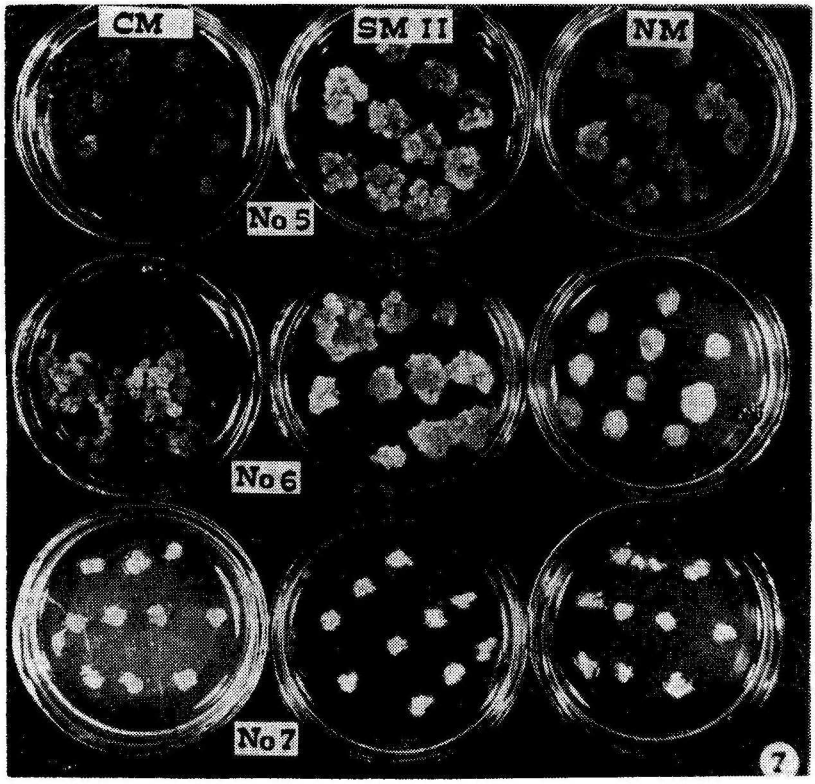

Fig. 7. Growth on the control (CM), selection (SM-II) and nitrate (NM) media of callus tissues of chlorate-resistant lines (lines No. 5 - 7) selected from dihaploid *S. tuberosum* cells radiated with gamma-rays at the dose of 5 kR

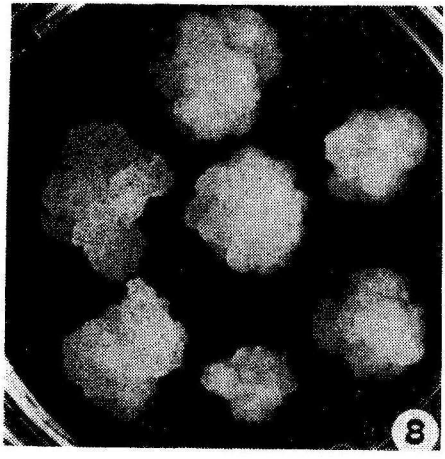

Fig. 8. Chlorate-resistant cell line No. 6 having callus tissue of a loose structure and white colour. on the selection medium SM-II

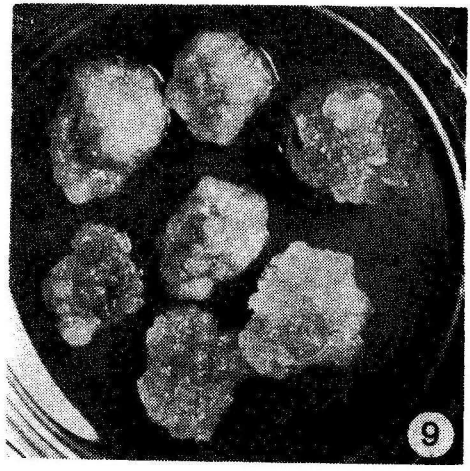

Fig. 9. Chlorate-resistant cell line No. 7 having callus tissue of a loose structure and grey colour, on the selection medium SM-II

Table 3. Callus growth coefficient (multiplicity of initial callus weight) of 3-year control lines (No. 1, No. 12) and chlorate-resistant (No. 2 - 11) *S. tuberosum* cell lines after 2-month culture on the medium: control (CM) (NH_4^+/NO_3^-; AA), selection (SM-II) (AA, $KClO_3$), and nitrate medium (NM) (NO_3^-)

Solanum tuberosum cells descending from lines	Dose of gamma-rays	Cell line	Callus growth coefficient of cell lines on the media			Colour of callus tissue in selected cell lines	Callus structure in selected cell lines
			CM	SM-II	NM		
	0 kR	1	25.40	3.38*	6.71	white	loose
	4 kR	8	1.64	1.85	0.92	white	tender
		9	1.60	1.84	0.89	white	tender
Pl. 620		2	1.39	8.77	0.89	lemonyellow	tender
$(2n-2x-24)$		3	7.92	7.29	3.78	white	compact
	5 kR	4	14.49	7.39	2.46	white	compact
		5	1.50	7.80	3.72	rust	tender
		6	11.99	7.67	4.20	white	loose
		7	1.43	1.66	0.91	dark-grey	loose
	0 kR	12	7.01	1.40	6.08	white	loose
m. 620 $(2n-x-12)$	4 kR	10	1.42	1.60	0.90	white	tender
		11	1.44	1.61	0.89	white	tender

* residual growth, lethal

to MS (1962); growth substance: 2.4 D — 2.0 mg/l, kinetin — 0.2 mg/l, NAA — 0.2 mg/l; sucrose — 30 g/l, agar — 8 g/l as in the CM medium.

After mutagenesis the plating efficiency (PE), survival (S) and mutagenesis efficiency (ME) indices were calculated for the colonies (Bartkowiak 1984).

RESULTS AND DISCUSSION

Irradiation of the cells decreased the plating efficiency and cell survival in the control medium (Table 1). Gamma-rays applied at the rate of 5 kR to dihaploid cells constituted a half-lethal dose (L_{D-50}) and resulted in the survival of 55 - 58% of the cells. Gamma-rays applied at the rate of 5 kR to monohaploid cells were a sublethal dose (L_{D-70}), since 37% of the cells survived, whereas the dose of 4 kR was half-lethal (L_{D-50}) and after its application 50% of the cells survived.

Results concerning PE and S of mono- and dihaploid cells of *Solanum tuberosum* in the SM-I medium of cultures incubated in the dark and in light are presented in Table 1. Like in the papers of many authors (Müller, Grafe 1975, 1978, Müller 1978, Horsch, Jones 1980, Steffen, Schieder 1983, 1984), the performed studies showed that in the cultures of dihaploid cells in the SM-I medium a small percentage of nonirradiated cells also survived. Plating efficiency in the cultures incubated in the dark was 12.13% and that in light was 9.62%. Survival indices calculated in relation to the control were 0.18 and 0.15 (Table 1). In the case of mutagenesis induction by gamma-rays the PE and S indices of mono- and dihaploid cells in the SM-I medium increased. However, in comparison to the PE and S indices of cells in the CM medium they were significantly lower (Table 1). The increase of plating

efficiency of radiated cells in the SM-I medium may indicate induced mutagenesis consisting in the loss of a nitrate reductase activity. As known from the works by other authors (Müller, Grafe 1975, 1978, Müller 1978, Horsch, Jones 1980, Steffen, Schieder 1983, 1984), cell resistance to chlorate is frequently a result of the nitrate reductase activity loss, but there may be also other reasons, the nature of which has not been known yet (Müller, Grafe 1978). Like in the work by Nielsen et al. (1985), PE and S of radiated cells in the SM-I medium were significantly lower than those in the CM medium (Table 1). This indicates that selection factor eliminates from cultures both nonmutated cells and cells having no physiological resistance to that factor. PE and S indices of cells in the SM-I medium in cultures incubated in light are lower than those in cultures incubated in the dark. It is possible that in this case light was a factor inducing reductase activity in the cells and that this enzyme in contact with the selection factor — $KClO_3$ reduced it to $KClO_2$ toxic to cells. In this connection selection pressure increased in relation to nonmutated cells. These results confirm the adaptional character of nitrate reductase. There is a close dependence of nitrate reductase activity on nitrate content in the substratum and on the presence of light (Duke, Duke 1984, Beevers, Hageman 1969).

The largest mutagenesis efficiency (ME — 0.65 - 0.67), when used in combination with the selection factor $KClO_3$, was obtained in the cultures of dihaploid *Solanum tuberosum* cells radiated with gamma-rays at the dose of 5 kR. In the cultures of monohaploid cells, however, in the case of their radiation with gamma-rays at the dose of 4 kR, mutagenesis efficiency was 0.30 - 0.33.

After 3 months of mutated colonies culture on the selection medium (SM-I) with the aim of testing the reaction of these cells resistance to chlorates the cell colonies (with variant frequency-VF presented in Table 1) grown on the SM-I medium were passaged on the subsequent selection medium (SM-II) containing $KClO_3$ and casein hydrolysate constituting the only source of nitrogen in the medium. When cell colonies were passaged on the SM-II medium many-fold, it was observed that resistance of many of them disappeared, which was expressed in the inability to develop on that medium (Table 2).

Cell colonies originating from nonradiated dihaploid cells, selected on the SM-I medium and passaged on the SM-II medium lost their resistance in the 5th passage. These cells presumably had a temporary physiological resistance to $KClO_3$. Cell colonies selected from monohaploid cells radiated with gamma-rays at the dose of 5 kR lost their ability to develop on the SM-II medium already in the 3rd passage. In the 10 passage, i. e. after 20-month selection, hardly 0.2 to 1% of inoculated colonies were observed. In the course of 3-year selection on the selection SM-II medium 10 chlorate-resistant cell lines were selected. Cell lines No. 8 and 9 descending from one cell colony (VF — 3.8×10^{-4}) were selected from 500 cell colonies originating from incubated in light dihaploid cells radiated with gamma-rays at the dose of 4 kR. 6 Cell lines No. 2 - 7 originated from separate 6 cell colonies were selected from 500 cell colonies descending from incubated in the dark dihaploid cells radiated with gamma-rays at the dose of 5 kR. Lines No. 2 and 3 from the

colony with VF — 6.3×10^{-4} selected from 500 cell colonies originating from cells incubated in light. Cell lines No. 10 and 11 descending from a single cell colony (VF — 1.4×10^{-4}) were selected from 500 cell colonies originating from incubated in light in monohaploid cells radiated with gamma-rays at the dose of 4 kR (Table 2). Like in the works of other authors (Müller, Grafe 1975, 1978, Müller 1978, Horsch, Jones 1980, Steffen, Schieder 1983, 1984) VF of cell colonies (out of which resistant cell lines were selected) is very low. The above cell lines differ in the callus colour from white (lines No. 3, 4, 6, 8 - 11) through lemon-yellow (line No. 2) and rust (line No. 5) to dark-grey (line No. 7). They also differ by the callus structure from soft and loose (lines No. 6 and 7) through tender (lines No. 2, 5, 8, 10, 11) to compact (lines No. 3 and 4). The selected chlorate-resistant cell lines (maintained in culture for over 3 years) differ from the control lines (lines No. 1 and 12) by the intensity of the callus tissue increase and by the way of its growth on both CM, SM-II and NM media (Table 3 and Fig. 1, 4, 7). The most stabilized with regard to these morphological characters are cell lines No. 2 - 7, originating from separate cell colonies of dihaploid *Solanum tuberosum* cells radiated with gamma-rays at the dose of 5 kR (Fig. 2, 3, 5, 6, 8, 9).

In Table 3 callus growth coefficients of 3-year control lines and chlorate resistant cell lines are presented.

The growth coefficient of callus on the CM medium shows, to what degree cell metabolism in the studied lines was discriminated as a result of radiation, by a comparison to the control lines. The growth coefficient on the SM-II medium, however, may reflect chlorate resistance, but only in the case, when it is compared to that on the CM medium of the same line. However, that comparison should be treated very carefully, since CM and SM-II media differed not only by the presence of chlorate in the SM-II, but also by casein hydrolysate concentration (CM — 0.5 g/l; SM-II — 2 g/l). The CM medium additionally contained ammonium nitrate (1650 mg/l), which was lacking in SM-II medium. Ammonium nitrate may be a source of nitrogen even for nitrate reductase-deficient lines, since it contains nitrogen in the ammonium form.

All radiated lines grew more weakly on the CM line than did the control line. This suggests a possibility of mutations in metabolism, probably manyfold in some lines. The callus growth coefficients on the CM and SM-II media in individual lines No. 8, 9, 10, 11, despite their chlorate-resistant are lower on the SM-II medium than the control lines, but growth on the CM medium is very discriminated and in fact does not differ from that on the SM-II medium.

Lines No. 4 and 6 have a higher growth coefficient on the CM medium and lower on the SM-II medium, but that may be a result of qualitative and quantitative differences in the nitrogen source in these media (point 1). Higher AA (amino acids) concentrations in the SM-II medium may inhibit growth, since AA frequently have an inbilitive effect on in vitro cultured tissues (Ratajczak, Buk 1976). Line No. 3 grows in the same way — perhaps the source of nitrogen in the CM and SM-II media is suitable to it and it is resistant to potassium chlorate — $KClO_3$. Lines No. 2 and 5 show a better growth at a higher AA level (contrary to lines

No. 4 and 6), but this growth may be also stimulated, which depends on the tissue properties. Potassium chlorate — $KClO_3$ in the SM-II medium may be an additional source of potassium, stimulating growth under the assumption of resistance to ClO_3^- ion.

Callus growth coefficients of cell lines No. 2 - 11 incubated on the SM-II medium are larger than those on the NM medium, whereas callus growth coefficients of control lines No. 1 and 12 on the SM-II medium are lower than those on the NM medium.

This indicates chlorate-resistance of lines No. 2 - 11. Since callus growth coefficients of chlorate-resistant cell lines No. 2 - 11 incubated on the NM medium are lower than those of control lines No. 1 and 12 incubated on the same medium, this may be indicative of discrimination of nitrate reductase activity in the selected cell lines in relation to nitrate reductase activity in the control lines.

Thus, on the basis of data in Tables 2 and 3 it may be inferred that all selected 10 cell lines (No. 2 - 11) already fit the term "stable variants" (— according to Müller 1978) — a criterion of referring cell lines to "stable variants" is survival of lines throughout one year on the $KClO_3$—containing medium followed by subculture on a nonselection medium through the next year and the fact that they do not restore characters of the "wild type" cells.

The mentioned cell lines (No. 2 - 11) have been grown on the SM-II medium containing potassium chlorate-$KClO_3$ for over 3 years. When being subcultured (passaged) for almost 2 years on the control CM medium, they did not display characters of the control lines (No. 1 and 12), so, they do not return to the properties of the "wild type" cells. These lines are stable with regard to the size of coefficients of the callus growth on the CM, SM-II and NM media and to other morphological characters, such as the colour, structure, the way and intensity of the callus tissue growth, i. e. traits by which chlorate-resistant cell lines differ from one another.

Regarding the kind of changes in nitrogen metabolism in the mentioned above lines, only after carrying out biochemical analyses it would be possible to say something on that subject. Detection of changes in the activity of enzymes participating in mineral nitrogen assimilation, and first of all, the detection of nitrate reductase deficiency will finally indicate the effectiveness of the applied radiation-induced mutagenesis and cell selection to chlorate.

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OTRZYMANIE LINII KOMÓRKOWYCH Z BRAKIEM LUB ZANIŻONĄ AKTYWNOŚCIĄ REDUKTAZY AZOTANOWEJ Z INKUBOWANYCH W SUSPENSJI KOMÓREK MONO- ($2n=x=12$) I DIHAPLOIDALNYCH ($2n=2x=24$) ROŚLIN *SOLANUM TUBEROSUM* L.

Streszczenie

Reduktaza azotanowa jest kluczowym enzymem odżywiania azotowego roślin i jego wydajność katalityczna może, w mniejszym lub większym stopniu, warunkować zdolność przyswajania azotu mineralnego u różnych gatunków roślin. Zalecenia agrotechniczne uprawy ziemniaka preferują nawożenie amonowe, co jest przesłanką, że u tego gatunku azotany są słabiej przyswajane i że manipulacje genetyczne polegające na wymianie reduktazy azotanowej na bardziej „wydajną" mogą przyczynić się do uzyskania odmian lepiej przyswajających azotany. Pierwszym krokiem w realizacji tych zamierzeń jest uzyskanie klonów pozbawionych własnej reduktazy azotanowej (NR^-), będących zatem dobrym biercą obcej NR. Metodą otrzymywania komórek z brakiem lub upośledzeniem aktywności reduktazy azotanowej, jest mutageneza komórek in vitro i ich selekcja klonalna na wysokie koncentracje chloranów (Müller, Grafe 1978).

W niniejszej pracy na drodze mutagenezy radiacyjnej inkubowanych w zawiesinie komórek (napromieniowanych promieniami gamma o mocy 500 r/min. w dawkach 4 i 5 kR) mono- i dihaploidalnych roślin *S. tuberosum*, a następnie ich selekcję klonalną na wysokie koncentracje chloranów (2,5 g/l $KClO_3$), wyselekcjonowano po raz pierwszy u ziemniaka 10 chloranoopornych linii komórkowych. Linie te przez 3 lata dobrze rozwijały się na pożywce selekcyjnej w obecności wysokiego stężenia chloranów. Wielokrotne pasażowanie tych komórek na pożywkę kontrolną nie spowodowało powrócenia ich właściwości do cech komórek typu dzikiego.

W związku z tym można je zaliczyć do wariantów stabilnych. Wyselekcjonowane linie komórkowe różniły się barwą, strukturą, sposobem i intensywnością wzrostu tkanki kulasa na pożywce kontrolnej i selekcyjnej, a także azotanowej, testującej aktywność reduktozy azotanowej. Wskaźniki wzrostu kallusa chloranoopornych linii komórkowych na pożywce

azotanowej wahały się od 0.89 - 4.72 i były one niższe od wskaźników wzrostu kallusa linii kontrolnych (6.08 - 6.71). Świadczy to o zaniżonej aktywności reduktazy azotanowej lub jej braku, w wyselekcjonowanych liniach komórkowych.

Wyselekcjonowane linie komórkowe mogą służyć również do badań nad rozszyfrowaniem mechanizmów regulujących proces przyswajania azotu mineralnego u ziemniaka.

ИЗОЛЯЦИЯ КЛЕТОЧНЫХ ЛИНИЙ БЕЗ ИНТРАТНОЙ РЕДУКТАЗЫ ИЛИ С ЕЁ ПОНИЖЕННОЙ АКТИВНОСТЬЮ ИЗ ИНКУБИРОВАННЫХ В СУСПЕНЗИИ КЛЕТОК МОНО-($2n=x=12$) И ДИГАПЛОИДНЫХ ($2n=2x=24$) РАСТЕНИЙ *SOLANUM TUBEROSUM* L.

Резюме

С целью получения клеточных линий in vitro, устойчивых к высоким концентрациям хлоратов, клетки моно- и дигаплоидных растений подвергались действию гамма-лучей при 500 об/мин в дозах 4 и 5 kR. Гамма-лучи в дозе 4 и 5 kR оказывали тормозящее воздействие на эффективность посева (PE) и выживаемость (S) клеток в контрольной среде (CM). В культурах дигаплоидных ($2n=2x=24$) клеток в селекционной среде (SM-I), содержащей хлораты, в небольшом проценте выживали также необлучённые клетки. Повышение эффективности посева облучённых клеток в селекционной среде может свидетельствовать об индуцированной мутагенезе, заключающейся в потери активности нитратной редуктазы в этих клетках. Из облучённых клеток выселекционировано 10 хлорато-устойчивых клеточных линий, которые в течение 3 лет хорошо развивались в селекционной среде при высокой концентрации хлоратов, а переносимые в течение одного года на контрольную среду, не вернули себе свойств дикого типа („wild type"), подлегая тем самым под критерий „stable variants" согласно Мюллеру (1978). Выселекционированные клеточные линии отличаются такими морфологическими признаками, как цвет, структура, способ и интенсивность роста ткани каллуса в питательной среде: контрольной (CM), селекционной (SM-II) и интратной (NM), проверяющей активность нитратной редуктазы, содержащей 3920 мг/л KNO_3. Показатели роста каллуса хлорато-устойчивых линий в среде HM колеблются от 0,89 до 4,72 и ниже показателей роста каллуса контрольных линий (6,08 - 6,71). Это свидетельствует о пониженной активности NR или ей отсутствии в селекционированных клеточных линиях.