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Cytogenetic Damage in Shallot (*Allium cepa*) Root Meristems Induced by Oil Industry "High-Density Brines"

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Abstract. Saturated water solutions of calcium chloride, calcium bromide (densities 1.30 kg dm^{-3} and 1.61 kg dm^{-3}, respectively) and their 1:1 mixture have been commonly used as oil industry "high-density brines." In our experiment they were added to tap water in amounts appropriate to achieve concentrations of 0.025, 0.05, 0.075, and 0.1 mol dm^{-3} to study their cytotoxic effect on the root tip cells of shallot (*Allium cepa* L. var. *ascalonicum*). All tested solutions in concentrations of 0.075 and 0.1 mol dm^{-3} caused significant inhibition of shallot root growth. $CaBr_2$ showed this effect in concentration 0.05 mol dm^{-3}. The investigated solutions in all concentrations applied decreased mitotic activity in root tip cells. The most of mitotic abnormalities were the consequence of spindle failure and chromosome stickiness. Furthermore, the cell microtubules were investigated by indirect immunofluorescence to confirm that most abnormalities observed were the consequence of spindle failure. The present study, as well as previously done *Lemna* tests and *Chlorella* tests showed that investigated samples have certain effects on plants, so constant control of their presence in the environment is needed.

Saturated water solutions of calcium chloride, calcium bromide (densities 1.30 kg dm^{-3} and 1.61 kg dm^{-3}, respectively) and their mixtures in different proportions, commercially prepared as "high-density brines," are commonly used during special operations in exploration and production of natural gas and crude oil. In accidental situations, certain amounts of these solutions could be released into the environment and pollute nearby fresh and ground waters and soil. Animal and plant organisms living close to oil wells could become exposed to increased concentration of salts.

So far the effects of high-density brines have been investigated mainly in aquatic species. Tkalec *et al.* (1998) investigated the influence of $CaCl_2$, $CaBr_2$, and their 1:1 mixture on duckweed (*Lemna minor* L.) growth. Four volume concentrations of these solutions were investigated, and it has been noticed that all three tested solutions in concentrations of 0.5%, 1.0%, and 1.5% (v/v) stimulated growth of *L. minor* while the highest concentration tested, 2.0% (v/v), caused inhibition. Lower concentrations of all three tested solutions had similar stimulative effect on the growth of green alga *Chlorella kessleri* Fott et Novák (Vidaković-Cifrek *et al.* 1999). On the other hand, among solutions of the highest concentrations (2.0% v/v), $CaBr_2$ showed the most prominent inhibitory effect. Mažuran *et al.* (1999) investigated the effect of these chemicals on the fecundity of the freshwater snail *Planorbarius corneus* L. The tested solutions containing bromide caused more prominent reduction of fecundity in comparison to $CaCl_2$ solutions.

Total plant growth and development depends on mitotic processes in plant meristematic regions. Cell division is a multistep process that demands the correct functioning of different cell organelles and products of many genes for its completion. Therefore, there are many potential targets for chemicals that can alter the efficiency of the process without totally disrupting it (Bond 1987).

The *Allium* test, used since the late 1930s and standardized in 1985 (Fiskesjö 1985) is a very sensitive and reliable plant assay method in environmental monitoring. It is based on the assessment of the toxic and genotoxic potential of chemicals in species of the genus *Allium* by measuring the mean root growth and by recording mitotic activity (mitotic index), mitotic abnormalities, and chromosomal aberrations in meristematic root tip cells.

There are few studies using the *Allium* in genotoxicity evaluation. Among them, the original form (Fiskesjö 1985) implies germination of onion bulbs in the tap water of known quality to a length of 1–2 cm and thereafter the specific treatment. A modification of the test implies root growth in tested liquids without pregrowth period in tap water (Fiskesjö 1985).

Microtubules (MTs) are structural components of the eukaryotic cytoskeleton and are involved in a number of processes, such as nuclear and cell division, organelle transport, cell motility, and maintenance of cell morphology. They are surprisingly dynamic protein structures present in four distinct arrays: preprophase band (PPB), mitotic apparatus, the phragmoplast, and the cortical MTs (Hepler *et al.* 1993). Their formation, structure and degradation are controlled by calcium (Hepler and Wayne 1985; Hepler 1992). A band of MTs known as PPB encircles the cortical cytoplasm before mitosis and

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Table 1. Chemical composition of $CaCl_2$ and $CaBr_2$ solutions of technical grade (densities 1,300 g dm^{-3} and 1,610 g dm^{-3}, respectively) commonly used as high-density brines

	Concentration	Ca^{2+}	Mg^{2+}	Zn^{2+}	Cl^-	Br^-
$CaCl_2$	mg dm^{-3}	170,000	6.0	1.8	301,350	—
	mol dm^{-3}	4.24	$2.47 \cdot 10^{-4}$	$2.75 \cdot 10^{-5}$	8.5	—
$CaBr_2$	mg dm^{-3}	218,000	195.0	6.1	—	867,500
	mol dm^{-3}	5.44	$8.02 \cdot 10^{-3}$	$9.33 \cdot 10^{-5}$	—	10.85

marks the zone where the new cell wall will be inserted (Palevitz 1987; Gunning and Sammut 1990). MTs and associated proteins are also components of mitotic spindle that ensure accurate segregation of chromosomes to daughter cells. MTs also comprise the most prominent elements within the phragmoplast.

In the present work the toxicity effects of high-density brines have been studied by measuring root growth and analyzing mitotic disturbances. To confirm if the mitotic abnormalities such as c-mitoses, multipolar anaphases, laggard chromosomes, vagrants, and polyploidy were the consequence of the spindle MT disruption, MTs were investigated by indirect immunofluorescence.

Materials and Methods

Test Organism

Approximately equal-sized and untreated bulbs (4-6 g) of shallot *Allium cepa* L. var. *ascalonicum* with chromosome complement of $2n = 2x = 16$, were used. After the harvest, bulbs were stored for a few months in the darkness under dry conditions at 6–10°C (winter rest) till the experiment has started.

Tested Chemicals

$CaCl_2$ and $CaBr_2$ solutions (densities 1.30 kg dm^{-3} and 1.61 kg dm^{-3}, respectively) and their 1:1 mixture were of technical grade. Atomic absorption spectrophotometry (ASTM 1995) and volumetric method (ASTM 1995) were used to determine an accurate amount of calcium chloride, calcium bromide, and some inorganic substances in these solutions (Table 1). Amounts of heavy metals (Cd, Cr, Ni, V, Fe, and Co) were under detectable levels. Detection limits for these metals were (mg dm^{-3}): Cd = 0.0005, Cr = 0.07, Ni = 0.008, V = 0.1, Fe = 0.005, and Co = 0.006.

In our experiment certain volumes of tested solutions were diluted with tap water to obtain the following final concentrations of $CaCl_2$, $CaBr_2$, and their 1:1 mixture: 0.025, 0.05, 0.075, and 0.1 mol dm^{-3}. Samples 1:1 mixture (1 part of $CaCl_2$:1 part of $CaBr_2$) contained both of the tested solutions in such volumes that final concentration of both salts was 0.025, 0.5, 0.075, or 0.1 mol dm^{-3}.

Toxicity Test Procedure

Root growth inhibition test as well as chromosome and mitotic aberration assay were carried out as described by Fiskesjö (1985, 1993, 1994).

The basal plates of the bulbs were immersed in tap water and allowed to produce roots until they reached the lengths of 1.5–2 cm (original form of the *Allium* test). The bulbs were then transferred to the tubes containing the tested solutions and exposed for 24 h. After that the microscopic slides were made. For each liquid, five bulbs were used.

For the study of root growth, the bulbs were placed directly on the top of the tubes filled with tested liquids (modified form of *Allium* test). Series of six onions were prepared for each concentration of tested solution. At day 5, the experiment was terminated, and the root lengths were measured for each single root. Exceptionally short roots (usually grown on the outer border of the root primordia) were ignored. Root lengths from six onions were used for calculation of mean value. Statistical analysis was carried out by Duncan's new multiple range test (Duncan 1955).

The experiments were performed at relatively constant room temperature (20°C) and protected from direct sunlight. Tap water, previously left overnight allowing chlorine to evaporate, was used as a negative control and for diluting the tested solutions. Tested solutions and control water were changed every day.

Preparation of Microscope Slides

In the original form of the *Allium* test the roots were cut off after 24 h of treatment and fixed in ethanol:acetic acid (3:1) for 24 h or more. After that, the roots were hydrolyzed in 1 M hydrochloric acid at 60°C for 10 min and stained with Schiff's reagent for 2 h. Root tip segments (1–1.5 mm starting from the root tip) were cut off and squashed in a drop of 45% acetic acid. For each concentration tested five root tips were squashed for five slides.

Permanent microscopic slides were prepared using liquid carbon dioxide (Sharma and Sharma 1972), air-dried overnight, and mounted in DePex mounting medium (Gurr). Permanent slides were analyzed under the light microscope Opton (Karl Zeiss, Germany) with an oil-immersion objective and a 1,000× magnification.

For each treatment and negative control 3,000–5,000 cells were scored. For the cytotoxicity assessment, mitotic index (MI), mitotic abnormalities (consequences of spindle disturbances), chromatin abnormalities (stickiness), and frequencies of mitotic phases were used as endpoints. The MI was calculated as the ratio between dividing cells and total number of cells scored. The frequency of each mitotic phase, mitotic abnormalities, and stickiness were expressed as the percentage in relation to the total number of cells in mitosis. Statistical analysis was performed using the Mann-Whitney *U* test (Daniel 1995).

Staining for Plant MTs

Root tips were prepared for indirect immunofluorescence localization of tubulin by a method described by Wick *et al.* (1981).

After the treatments, 1.5-cm-long root tips were fixed in 4% paraformaldehyde (PFA) in 0.1 mol dm^{-3} piperazine-N,N′-bis (2-ethanesulfonic acid) (PIPES) buffer containing 3 mol m^{-3} ethylene glycol-

Table 2. Effect of $CaCl_2$, $CaBr_2$, and their 1:1 mixture on shallot root growth recorded after 5 days of treatment in modified *Allium* test

Concentration (mol dm^{-3})	Root Length (mm)		
	$CaCl_2$	$CaBr_2$	$CaCl_2$ + $CaBr_2$ (1:1)
0.0 (control)	32.80 ± 2.66 A	31.01 ± 2.85 A	33.06 ± 3.57 A
0.025	32.98 ± 3.92 A, a	26.54 ± 5.74 B, bc	29.72 ± 4.23 A, abc
0.050	30.81 ± 2.17 A, ab	27.96 ± 2.45 AB, abc	29.62 ± 2.32 A, abc
0.075	26.12 ± 4.10 B, cd	16.96 ± 1.33 C, e	23.36 ± 4.90 B, d
0.100	14.94 ± 1.95 C, ef	8.45 ± 0.51 D, g	12.37 ± 0.91 C, fg

Each value is mean ± SD of average root length taken from six bulbs. Values within the column are compared to evaluate the effect of different concentrations of the same sample and marked with the uppercase letters. Lowercase letters enable the comparison of the effects of different samples (comparison of data within the whole table). Values marked with the same letter are not significantly different at the $P < 0.05$ level according to Duncan's new multiple range test.

bis(β-aminoethyl ether) (EGTA), pH 9, for 1 h at room temperature. Root segments were subsequently washed in the same buffer three times for 20 min and macerated in the enzyme solution containing 0.3% Pectolyase Y-23 (Seishin Pharmaceutical, Koamicho, Niconbashi, Tokyo), 0.1% Cellulase Onozuka RS (Yakult Honsha, Tokyo), 5 mol m^{-3} $CaCl_2$, and 5 mol m^{-3} 2(N-morpholino) ethanesulfonic acid (MES), pH 5.5, at 37°C for 1 h. Following enzyme treatment, the root segments were washed twice in PIPES-EGTA buffer and prepared for antibody treatment. Isolated root tips were placed on a microslide under a coverslip. The coverslip was tapped gently to release the cells from the root tissue. Coverslips were removed carefully, and the slides with the liberated cells allowed to dry overnight. Afterward, slides were rinsed in 10% phosphate buffered saline (PBS) flooded with 0.5% Triton X-100 (w/v) in PBS for 10 min, and washed again in PBS. The cells were incubated with monoclonal antibody (rat IgG) against tubulin (Sera-Lab, Crawley Down, UK) diluted 1:300 in PBS, at 37°C for 45 min. After washing in PBS, the cells were stained with fluorescein isothiocyanate–labeled goat-anti rat IgG (Southern Biotech, Birmingham, UK) diluted 1:60 in PBS at 37°C for 45 min. After washing in PBS, the nuclei were stained for 5 min with 10 μg ml^{-1} Hoechst 33342 (Sigma). After they were washed in PBS, the cells were mounted in Citifluor mounting medium (Agar Scientific, Stansted, UK). Coverslips were sealed to the microslide with nail polish. The slides were examined in a microscope equipped with epifluorescence illumination (Opton Axioplan, Germany). Photographs were taken with 100× objective on Ilford XP-2 black and white film (400 ASA).

Results

$CaCl_2$, $CaBr_2$, and their 1:1 mixture in concentrations of 0.075 and 0.1 mol dm^{-3} significantly inhibited root growth in comparison with the control (Table 2). The most prominent inhibitory effect was observed after the treatment with $CaBr_2$ (all concentrations tested).

The fraction of cells in mitosis (expressed as mitotic index) after the treatment with all tested samples was significantly lower than in control (Figure 1). After the treatment with $CaCl_2$, MI decreased with the increase of tested salts' concentration. On the other hand, the concentration 0.05 mol dm^{-3} of $CaBr_2$ and the 1:1 mixture caused higher MI values than the concentration of 0.025 mol dm^{-3} (Figure 1).

The rhythm of mitotic division was also changed. Compared with control, the frequency of prophase slightly increased after all treatments, except for the highest concentration (0.1 mol dm^{-3}) of all samples as well as for the treatment with mixture in concentrations of 0.05 and 0.075 mol dm^{-3}. The highest concentration caused a slight increase in metaphase figures, but such effect is also caused by the lowest concentration of mixture (Table 3).

As shown previously (Figure 1), concentrations of 0.025, 0.05, 0.075, mol dm^{-3} $CaCl_2$ as well as 0.025 and 0.05 mol dm^{-3} $CaBr_2$ and the 1:1 mixture inhibited mitotic activity but slightly disturbed cell division. The higher concentrations significantly inhibited mitotic activity with simultaneous appearance of mitotic disturbances (Table 4).

The most frequent abnormalities were those connected with spindle failure (c-mitosis, multipolar anaphases, lagging chromosomes, vagrants, polyploidy) and chromosome stickiness (Table 4, Figure 2a, b, and c). Besides that, the drastic disturbances of chromosome morphology after the treatment with $CaCl_2$ (concentration 0.025 mol dm^{-3}) were found (Figure 2d), which could be the consequence of chromosome damage or eroded chromatine.

As the majority of metaphase and anaphase abnormalities noticed could be the consequence of disturbed function of cell MTs, the distribution of MTs in root-tip cells was identified by tubulin immunodetection. It has been confirmed that mitotic irregularities (such as multipolar anaphase, lagging chromosomes, and vagrants) were the consequence of spindle failure. Besides regularly developed mitotic spindles (Figure 3a), destroyed or incompletely developed spindles were observed (Figure 3b). Numerous PPBs were observed, some of which were oriented longitudinally (Figure 3c), especially after the treatment with $CaBr_2$ in concentrations of 0.075 and 0.1 mol dm^{-3}. Some PPBs appeared broken, incompletely developed, or bifurcated (Figure 3d), and some were placed asymmetrically.

Discussion

Our results indicate that tested solutions affect the cell division process in shallot root tip cells. It is well known that calcium

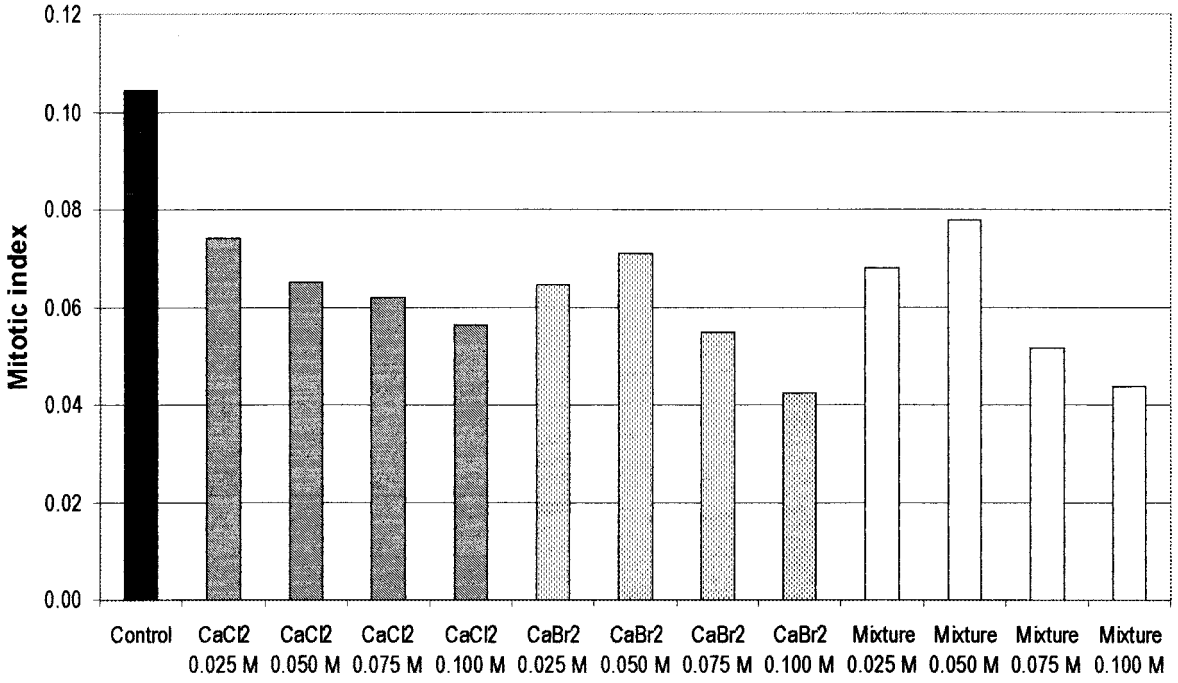

Fig. 1. Mitotic index in the root tip cells of shallot treated with $CaCl_2$, $CaBr_2$, and their 1:1 mixture for 24 h. Statistical analysis by Mann-Whitney *U* test showed significantly different mitotic activity ($p < 0.05$) between the control and treated root tip cells

Table 3. Distribution of mitotic phases in shallot root tip cells treated for 24 h with $CaCl_2$, $CaBr_2$, and their 1:1 mixture as well as in the control cells

Sample	Concentration (mol dm^{-3})	Percentage of Mitotic Phases (%)			
		Prophase	Metaphase	Anaphase	Telophase
Control		49.8	14.3	12.2	23.7
$CaCl_2$	0.025	60.3	15.1	6.5	18.2
	0.050	58.1	19.1	11.2	11.6
	0.075	57.2	18.4	9.7	14.7
	0.100	49.4	21.9	12.7	16.0
$CaBr_2$	0.025	61.8	14.8	8.3	15.1
	0.050	54.0	15.3	12.0	18.8
	0.075	58.8	13.4	7.3	20.4
	0.100	48.5	23.0	11.5	17.0
Mixture (1:1)	0.025	60.1	17.9	7.2	14.9
	0.050	50.5	14.3	12.0	23.2
	0.075	52.2	15.9	9.4	22.5
	0.100	50.3	18.5	11.6	19.7

ions act as regulator of the mitotic events: They control MTs assembly/disassembly, chromatin condensation/decondensation process, membrane transformations, chromosome separation, and transport (reviewed by Hepler 1992). Spindle MTs can be disassembled both *in vivo* and *in vitro* by elevated level of calcium, whereas its low level lead to polymerization (Hepler 1992). Low concentration of free calcium in cytoplasm (0.1–0.2 μM) is essential for prevention of phosphorus precipitation, competition with Mg^{2+} for binding sites and functioning of calcium as second messenger (Marschner 1988). Because elevated levels of calcium cause MTs depolymerization (Hepler and Wayne 1985; Hepler 1992) it is possible that this ion, present in all investigated samples, could influence mitosis in shallot root tips. Results of chromosome aberration analysis show that all chemicals tested induced aberrations that appeared as a consequence of MTs disturbances. Mitotic spindles with disrupted or incompletely developed MTs correlate with cell division abnormalities found, such as c-mitoses, multipolar anaphases, lagging chromosomes, vagrant chromosomes, and polyploidy (Figure 2). Slightly increased percentage of metaphase cells after the treatment with the concentration 0.1 mol dm^{-3} (Table 3) could be the consequence of metaphase arrest caused by tested samples, and it leads to the conclusion that this treatment blocked the progress of mitosis in metaphase. Prolonged metaphase after increase of extracellular calcium concentration has already been described by other authors

Table 4. Spindle disturbances and chromosome stickiness in the root tip cells of shallot treated with tested solutions for 24 h

Sample	Concentration (mol dm^{-3})	Number of Mitotic Cells	Number of Mitotic Aberrations		Percentage of Mitotic Aberrations	
			SD	CS	SD	CS
Control		476	4	0	0.84	0
$CaCl_2$	0.025	292	8	0	2.74	0
	0.050	277	7	3	2.53	1.08
	0.075	320	6	1	1.88	0.31
	0.100	237	17	0	7.17	0
$CaBr_2$	0.025	458	9	0	1.97	0
	0.050	485	8	2	1.65	0.41
	0.075	313	10	0	3.19	0
	0.100	165	10	0	6.06	0
Mixture (1:1)	0.025	531	14	0	2.64	0
	0.050	699	25	3	3.58	0.43
	0.075	395	20	0	5.06	0
	0.100	173	7	1	4.05	0.58

SD, spindle disturbances (c-mitosis, disturbed anaphases, lagging chromosomes, vagrants, polyploidy).
CS, chromosome stickiness.

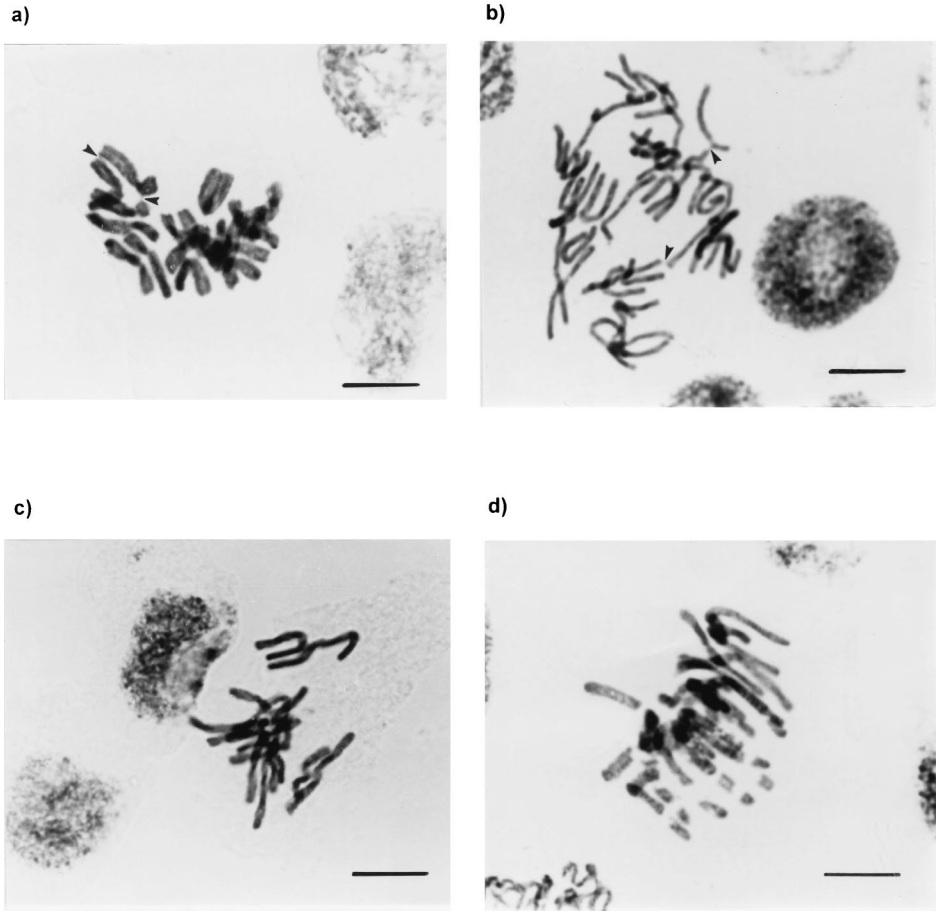

Fig. 2. The most abundant aberrations in onion root-tip cells after the treatment with high-density brines solutions. Root-tips were stained with Schiff's reagent. Magnification bars represent 10 μm. (a) C-mitosis and stickiness (arrows) after the treatment with 0.025 mol dm^{-3} $CaCl_2$. (b) Disturbed anaphase and stickiness (arrows) after the treatment with $CaCl_2$, concentration 0.025 mol dm^{-3}. (c) Vagrant chromosomes after the treatment with $CaCl_2$ and $CaBr_2$ (1:1 mixture) concentration 0.05 mol dm^{-3}. (d) Drastic disturbance of chromosome morphology has been observed after the treatment with $CaCl_2$, concentration 0.025 mol dm^{-3}

(Hepler and Wayne 1985). To study the effect of copper and different amounts of calcium in tap water, Fiskesjö (1981) used control water sample containing 72 ml/L Ca (1.796 mM). That sample caused delayed anaphases (3.0%) and vagrant chromosomes (0.2%). In the same paper, the growth retarding effect of calcium in concentration 350 mg/L (8.732 mM) was confirmed.

Environmental signals as well as increased level of calcium in apoplasm (Marschner 1988; Kauss 1987) can activate calcium channels in the membranes and increase calcium influx and cytosolic free calcium concentration. Zhang *et al.* (1990, 1992) in *Tradescantia* stamen hair cells showed that increase in calcium concentration within a limited physiological range

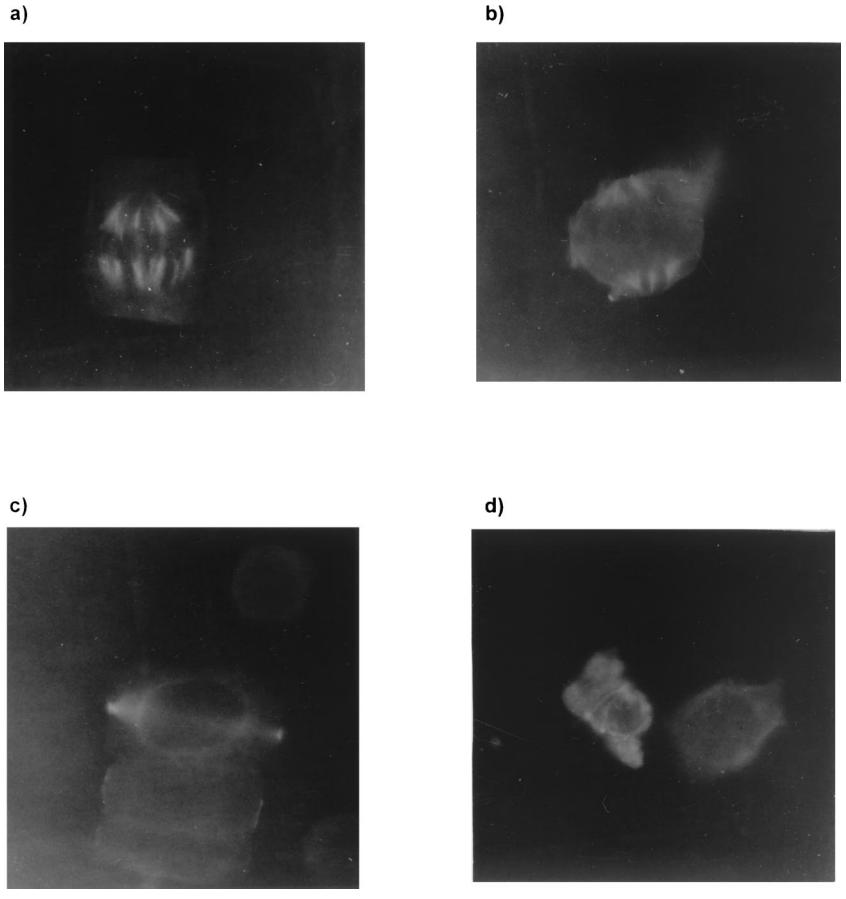

Fig. 3. FITC-fluorescence of MTs in shallot root tip cells after the treatment with high-density brines solutions. Magnification: 800×. (a) Mitotic spindle regularly developed (control). (b) Incompletely developed mitotic spindle (0.075 mol dm^{-3} $CaCl_2$). (c) Longitudinally oriented PPB ($CaBr_2$, 0.075 mol dm^{-3}). (d) Bifurcated PPB (0.075 mol dm^{-3} $CaCl_2$)

(0.8–1.0 μM) can stimulate chromosome motion and promote anaphase through a relatively small degree of kinetochore MT depolymerization. On the other hand, if calcium concentration rises above this range, an extensive degradation of kinetochore MTs occurs and chromosome motion will slow or stop (Zhang *et al.* 1992). As a consequence of slow chromosome movement that fails to segregate, the progress of mitosis in metaphase can also be blocked. In the cytosol the principal targets of Ca signals are Ca-binding proteins. The most common is calmodulin, associated with the mitotic spindle and phragmoplast at cytokinesis (Wick *et al.* 1985). An increased level of calcium, presumably acting through calmodulin, stimulates the depolymerization of spindle MTs (Kiehart 1981). So if elevated calcium concentration facilitates the depolymerization of MTs, one might predict disruption of the spindle structure. Although Zhang *et al.* (1990) expect that the high calcium concentration depolymerizes the spindle MTs in *Tradescantia* stamen hair cells, followed by complete disruption of the spindle structure and disorganization of chromosomes, they nevertheless fail to observe any gross disruption of the mitotic apparatus leading to rearrangement of the chromosomes. The elevated calcium thus affects spindle structure quite differently from colchicine and other MT agents. In their further investigations with fluorescent-labeled tubulin Zhang *et al.* (1992) noticed a reduction in the fluorescence intensity of the spindle fibers after injection of calcium. It confirmed that high level of free calcium in a dividing cell actually alters chromosome motion and spindle MT structure. Our data show destroyed or incompletely developed spindles after the treatment with calcium-containing solutions (Figure 3b). So it could lead to conclusion that elevated level of calcium could have such effect in *Allium* root tip cells.

In the cells treated with tested solutions, longitudinally oriented PPBs were noticed (Figure 3c). As a consequence of such PPBs, the spindles would be transversely oriented appearing to converge on the plane of the future phragmoplast. The reorientation of the future phragmoplast from transverse to the longitudinal can result in changes of the growth polarity of the elongating root cells and radial expansion of the cortical parenchyma. The possible consequences of PPB reorientation have been studied by Pavlica *et al.* (1998). They noticed the longitudinally oriented PPBs after the treatment of shallot root cells with the herbicide trifluralin. Dinitroaniline herbicides such as trifluralin and oryzalin, as well as 2,4-D, inhibited MT polymerization by binding to tubulin (Grant 1982; Morejohn *et al.* 1987; Morejohn 1991; Pavlica *et al.* 1998), but an increased content of calcium ions causes such effect through regulation of MT polymerization/depolymerization.

Misra (1982) studied the effects of $Ca(NO_3)_2$ and $CaCl_2$ on *A. cepa* chromosomes. Among the chromosomal changes the most frequently noticed were stickiness as well as chromosomal breaks and chromatin degeneration. According to Fiskesjö and Levan (1993) stickiness is a common sign of toxic influence on the chromosomes and is probably an irreversible effect. It is a type of abnormality that involves proteinaceous

matrix of chromatin material rather than DNA itself. In our work chromosome breaks and chromatin degeneration (Figure 2d) were very rarely noticed, but in Misra's work they appeared often after the treatment with higher concentrations of calcium salts (0.084 and 0.105 mol dm^{-3}) lasting 24 h or longer.

Our attention can now be turned to the possible role of other two constituents of tested samples—chloride and bromide. High concentration of chloride restricts the root growth (Combrink *et al.* 1995), but we did not find any data about effect of chloride salts, except for the already mentioned work of Misra (1982). Bromine is not an essential element for plant growth, but it can be absorbed by plant roots (Bowman *et al.* 1997). The same authors investigated the influence of KBr on germination and growth of crop seeds. Bromide added as KBr at concentrations up to 500 mg dm^{-3} did not affect the germination of any crop. It had little effect on crop growth at concentrations up to 1,000 mg dm^{-3}. Sharma (1990) reported that bromine caused metaphase arrest and appearance of laggard chromosomes in *A. cepa* that could lead to aneuploidy.

Chemical analysis of high-density brines showed certain amounts of magnesium and zinc (Table 1). Because the tested solutions contained these elements in very low concentrations, we do not expect them to have any possible effect on the root tip cells. However, Vujević *et al.* (2000) investigated the effect of $CaCl_2$ and $CaBr_2$, (analytical as well as technical grade) and compared their effects. For that purpose, the *Lemna* test was used and only minor differences caused by treatment with chemicals of different degree of purity were noticed. It supports the hypothesis that observed effects were caused by main constituents of high density brines (Ca^{2+}, Cl^-, and Br^-) and was not caused by impurities present in chemicals of technical grade.

The effects of tested salts on cell division may result from the disturbance of osmotic relationships. Although some researchers reported decrease of growth in osmotically stressed roots, the results of Seijo *et al.* (1997) showed that moderate osmotic stress applied to the roots of pea seedlings (*e.g.*, $\psi_\pi = -0.65$ MPa) promoted growth, but it gradually decreased when roots grew at lower osmotic potentials. In our work, even the highest concentration tested has osmotic potential not lower than -0.65 MPa, so we can conclude that osmotic potential itself did not cause the reduction of root growth.

Keeping in mind all the data mentioned, it could be concluded that almost all of mitotic disturbances noticed after the treatment with tested high-density brines solutions were the consequence of effects on the cell MTs.

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