Botanica SERBICA

(2020) 44 (2): 175-182

DOI: https://doi.org/10.2298/BOTSERB2002175S
journal homepage: botanicaserbica.bio.bg.ac.rs

Original Scientific Paper

Concentration- and time-dependent effects of strontium on *Lens culinaris* Medik.

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ABSTRACT:
This study investigates how strontium (Sr) ions act on meristematic root tip cells of lentil (*Lens culinaris*) with changing parameters (time and concentration). Plant seeds were exposed to both a standard solution of Sr for different lengths of time (1/4, 1/2, 1, 2, 4, 8, 12, 16, 20 and 24 hours) at a fixed concentration of 1.0 molL^{-1} (M) and Sr ions at various concentrations (0.05, 0.1, 0.25, 0.5 and 1.0 M) for a certain length of time (12 hours). The seeds treated with Sr were made to sprout and microscopic examination focused on the root tips. The aim of microscopic examination was to clarify chromosomal abnormalities of cell division. Microscopic examination showed that various abnormalities occurred in cells of the seedlings, abnormalities such as chromosome adherence, chromosome breakings, bridge chromosomes, chromosome dispersion, chromosome shrinking, fish bones and ring chromosomes. Those abnormalities were detected several times for each treatment depending on the different periods and concentrations. Adsorption and absorption of Sr inside lentil seeds were detected by the spectroscopic method. Removed and excess amounts of Sr ions were found by spectroscopic determination. Statistical evaluation of the results was used in order to reveal the differences and similarities. The results showed that while there is a positive correlation with the concentration parameter, there is a negative correlation with the time parameter. Over 90% of Sr was removed from the solution during 12-hour exposure. Lentil seeds can be accepted as good bioaccumulators of Sr ions only for an exposure period shorter than 12 hours at an Sr concentration of 1 M.

Keywords:
chromosome abnormalities, bioaccumulator, lentil, cytogenetic effects

UDC: 582.736.3: 581.162.1

Received: 31 December 2019
Revision accepted: 23 April 2020

INTRODUCTION

Cytogenetic studies are vital to an understanding of plant responses to outside influences (Saxena *et al.* 2009; Özdemir *et al.* 2015; Garlich *et al.* 2016). Andronic (2012) examined the effect of viruses on tomato plants and DNA. Cytogenetic effects of metals, especially heavy metals, are in the focus of research efforts because of the huge amount of environmental pollution caused by metal-based industries (Aksoy & Deveci 2012; Pesnya 2013; Sepet *et al.* 2014; Özdemir *et al.* 2015). Those pollutants can have a direct effect on plant cytogenetics by causing small or large chromosomal changes (Gupta 2006). Plants uptake metals from soils and accumulate them in different parts, from roots to leaves (Baranowska-Morek & Wierzbicka 2004; Maestri *et al.* 2010; Sepet *et al.* 2014).

Metals have a positive effect on plant growth until their concentration reaches a certain point (Tangahu *et al.* 2011; Bhargava *et al.* 2012; Çanli 2018; Sabovljević

et al. 2018), and they also change the structure of chromosomes inside the plant (Özdemir *et al.* 2015; Çanli 2018). In several investigations, six types of alteration in chromosomes were named, viz., bridges, C-mitosis, fragments, laggards, multipolarity and stickiness (Kuchy *et al.* 2016; Özkul *et al.* 2016). Moreover, Utsunomiya *et al.* (2002) described abnormalities such as chromosome segregation, absence of cytokinesis, cytomixis, cell fusion, irregular cell shape, chromosome bridges and genomic separation. According to Özdemir *et al.* (2012), chromosome abnormalities are classified into three categories: chromosome breaking, chromosome adherence and chromosome dispersion. There is another more detailed classification that includes fish bone in addition to chromosome adherence (Özdemir *et al.* 2015). When chromosomes do not detach at the end of the metaphase and remain adherent as indicated by Pekol *et al.* (2016), they look sticky (Wang *et al.* 2014). This appearance is refered to as "fish bone" in the latter study. The cytogenetic effects of metals have remained in the focus of recent research efforts (Kiran & Şahin 2005; Janas *et al.* 2010; Sepet *et al.* 2014; Özdemir *et al.* 2015).

Strontium (Sr) is a metal element with a bright white colour commonly found in the Earth's crust (about 0.04% of the Earth's crust) (MacMillan *et al.* 2000). Previous investigations treating the mechanism of metal pollution in plants demonstrated that Sr can be easily absorbed by them (Çelik *et al.* 2005; Manzatu *et al.* 2015; Lemtiri *et al.* 2016). Strontium can replace calcium in some foods and be accumulated in human bones and teeth (Burr & Allen 2019; Katzenberg & Grauer 2019). Seregin & Kozhevnikova (2005) and Kudo *et al.* (2015) found that metals inhibit maize seed germination in the following order: Mg > Cd > Ni ≈ Pb > Sr. Wójciak–Kosior *et al.* (2019) studied the effect of Sr on soybean sprouts in low concentrations (between 1 and 10 mM) for 72 hours. They found the best germination at 5 and 10 mM concentrations of Sr.

Accumulation of metals in plants has been of great interest to of researchers (Jakovljević *et al.* 2019; Prica *et al.* 2019) because there is no need to spend more money on other removing techniques. Jakovljević *et al.* (2019) focused on the heavy metal tolerance of *Pontechium maculatum* in order to gauge its usefulness as an accumulator. In addition, Prica *et al.* (2019) investigated bioaccumulation of heavy metals in *Phragmites australis*. Sevik *et al.* (2020) were interested in determination of lead (Pb) and magnesium (Mg) in some landscape plants. Burger & Lichtscheidl (2019) investigated the reactions of plants to stable and radioactive Sr isotopes.

Lentil is interesting because it is able to accumulate metals and show effects of metal toxicity on its growth processes in every part (Talukdar 2013). Talukdar (2013) used lentil to understand the effects of arsenic (As) accumulation on the plant, but his work only involved exposure of the plant to solid sodium arsenate. He found that As is accumulated in different amounts in eight genotypes of lentil. Janas *et al.* (2010) pointed out that accumulation of copper ions occurs in vacuoles of lentil and its root cell walls. Çanli (2018) focused on determining aberrations in *Lens culinaris* Medik. caused by barium (Ba) and vanadium (V) ions. Aksoy Dalgiç & Dane (2007) and Aksoy Dalgiç *et al.* (2007) studied the effect of fusilade at four different concentrations on seed germination, mitotic frequency, α-amylase activity and shoot and root growth in *L. culinaris*. Even though there are several studies treating *L. culinaris* (Talukdar 2013; Sepet *et al.* 2014; Hossain *et al.* 2019; Laskar *et al.* 2019; Mitra & Paul 2020; Pandey & Sengar 2020), no effort has been made to understand the effect of Sr on chromosomal change in *L. culinaris*, especially at such a high Sr concentration.

In this study, we investigated how Sr treatment of *Lens culinaris* for different lengths of time and at different concentrations of Sr affected root tip cells of the plant. The main objectives of the study were to clarify the influence of Sr on cell division, ascertain chromosomal abnormalities in the treated plants and establish the nature of Sr bioaccumulation.

MATERIAL AND METHODS

All experiments were carried out in laboratory conditions. Lentil seeds, which have 2n = 14 chromosomes, were used to clarify the effects of Sr in the study. Meristematic cells located in the root tips of lentil seedlings were prepared for microscopic examination of chromosomes as explained in Singh (2003).

Ten seeds were used for each treatment and as a control group. The seeds were chosen for being sound, plump and equal-sized. They were left in 10% sodium hypochlorite for 10 minutes in order to prevent contamination (Sepet *et al.* 2014). To remove excessive hypochlorite, the seeds were washed five times with distilled water, after which they were dried on filter paper at 25°C for 1 hour. A 1.0 M standard solution of Sr, which was prepared from strontium nitrate (Sigma Aldrich), was applied to the seeds for different lengths of time. Using an overdose of Sr helped us to shorten the exposure time needed to see abnormalities occurring in the cells.

Seeds were kept in the Sr standard solution at fixed concentration of 1.0 mol L^{-1} (M) for 1/4, 1/2, 1, 2, 4, 8, 12, 16, 20 and 24 hours, a batch being left in purified water for 24 hours as a control. To establish influence of the concentration parameter, seeds were kept at different concentrations of Sr (0.05, 0.1, 0.25, 0.5 and 1.0 M) for 12 hours, the control group being left in water for the same period of time. Distilled water was then used to remove the metal solution remaining on the seeds, which were allowed to germinate in petri dishes at 20–25°C. For fixation, root tips 1.5-2 cm long were cut and left in a fixative consisting of a solution of ethyl alcohol and glacial acetic acid in a ratio of 3:1. Stock root tips were

stained by the Feulgen method (Murray *et al.* 1992) to get them ready for more detailed examination. In microscopic determination of cell abnormalities, homologous areas were preferred. The number of mitotic cells was determined. The mitotic index was calculated based on the frequency of division in cell images obtained under a microscope. The occurrence and frequency of chromosomal abnormalities in the cells were established by hand counting from the microscope images. A Leica DM 3000 microscope with motorised screening was used for photographing the preparations.

Spectroscopic determination of Sr ions was accomplished by flame atomic absorption spectroscopy (AAS) using a PG-990 atomic absorption spectrometer (PG Instruments, United Kingdom). After calibration of the atomic absorption spectrometer with reference material, five standard solutions of $SrNO_3$ were prepared to determine the calibration equation between absorbance and concentration, after which all the exposed solutions were administered in flames to the machine. With reference to the standard solutions, all calculations were converted automatically by the atomic absorption spectrometer in light of the concentration-absorbance graph. The remaining statistical procedures such as calculation of the Pearson correlation between variables were carried out using the SPSS 22 program package. The percentage of adsorbed Sr was calculated using the following equation:

$$\% \text{ adsorbed Sr} = \frac{(\text{amount of Sr to which seeds were exposed} - \text{amount of Sr left in solution}) \times 100}{\text{amount of Sr to which seeds were exposed}}$$

RESULTS

The relationship between the concentration of Sr to which seeds were exposed and adsorbed amount of it is presented in Fig. 1. Absorption of Sr ions from the seeds increased for up to 4 hours, in parallel with decrease in the mitotic index (Table 1). At 8 hours of exposure, while absorption decreased, the mitotic index increased again. Germination was not observed after 8 hours of application.

Time parameter. The following anomalies were observed: treatment with a standard solution for different lengths of time resulted in increasing mitotic cell division in the seedlings. This cell division reached the highest level in the case of 1/4-hour treatment. Between ½ and 4 hours, the rate of mitotic cell division decreased. It began to rise again after 8 hours of treatment, but no division was observed after 12 hours. The control group showed more mitotic cell division in seedlings at every stage of treatment (Table 1).

Maximum absorption of Sr from the solution was recorded after half an hour (95%), and the lowest percentage was observed after 12 hours (81%) (Table 2). Correlation between exposure time and the amount of Sr left in the solution was r = -0.9218.

In addition to the changes in cell division, there were also several abnormalities detected in the cells after treatment with Sr (Fig. 2). Depending on their shape, they can be described as bridge chromosomes, chromosomal adherence, chromosome breaking, chromosome dispersions, chromosome shrinking, fish bones and ring chromosomes (Figs. 2 & 3). All the abnormalities were counted separately from other abnormality types, and the percentages of their occurrence were calculated for each abnormality. In all counted cells, fish bone abnormalities and bridge chromosomes were seen with the highest percentages at 1 hour (36.40 and 18.20%, respectively). For chromosome dispersions and chromosome breakings, the highest percentages of counted cells exhibiting them were detected after 4-hour exposure (30.40 and 15.20%, respectively). The highest percentages of counted cells with chromosome adherences and chromosome shrinking were recorded after 2 hours of exposure (26.88 and 40.32%, respectively). The ring chromosome abnormality was seen only in the cases of 2-hour and 8-hour exposures (in 13.44 and 11.74% of the counted cells, respectively). Except for chromosome breaking and ring chromosome abnormalities, all abnormalities were found at all exposure times.

Chromosome shrinking nearly reached the level of up to half of the counted cells after 2 hours of exposure (40.32%). It was also high in the case of 8-hour treatment

Table 1. Mitotic index of root tip cells of *Lens culinaris* at different times of exposure to Sr in a concentration of 1M.

Time (Hours)	Mitotic Index ± *S.D.	Time (Hours)	Mitotic Index ± *S.D.
1/4	18.80±4.75	8	8.42±1.94
1/2	16.09±5.10	12	-
1	11.22±3.82	16	-
2	7.88±4.14	20	-
4	6.72±3.43	24	-
Control Group	**19.12±6.35**		

Table 2. Adsorbtion of Sr by seeds of *L. culinaris* with changing exposure time as detected by flame-AAS (1.00 M).

Exposure time (h)	Concentration of Sr to which seeds were exposed (M)	Amount of Sr left in the solution (M)	Adsorbtion of Sr (%)
½	1	0.05	95
1	1	0.06	94
2	1	0.06	94
4	1	0.06	94
8	1	0.09	91
12	1	0.19	81

Table 3. Adsorbtion of Sr (%) by seeds of *L. culinaris* with changing concentration (at 12 h).

Concentration of Sr to which seeds were exposed (mg Sr/L)	Adsorbtion of Sr (%)
4.5	6.67
9.0	52.2
22.5	49.6
45	88.5
90	93.8

with Sr (in almost 35% of the counted cells). There were also fish bone abnormalities after 1 hour of treatment (in over over 35% of the counted cells). For the other abnormalities, the percentages of counted cells exhibiting them varied between 5 and 30%. The fish bone type abnormality was recorded in between 19 and 37% of the counted cells.

Concentration parameter. At the end of 12 hours of application at different concentrations, the amounts of the solutions which were absorbed by the seeds and the amounts remaining in solution are given in Table 3. The maximum absorption was recorded at a concentration of 1.00 M (93.8%), while the lowest absorption was at 0.05 M (6.67%). There was a direct relationship between the concentrations. The correlation between Sr concentration and the adsorbed amount of Sr was r = 0.9964.

DISCUSSION

The results indicate that Sr stress caused certain abnormalities such as fish bones, chromosome adherence, chromosome dispersions, bridge chromosomes, chromosome breaking, chromosome shrinking and ring chromosomes (Fig. 3). Sepet *et al.* (2014) found chromosome dispersions to be the abnormality with the highest frequency in lentil seeds (occurring in 52% of

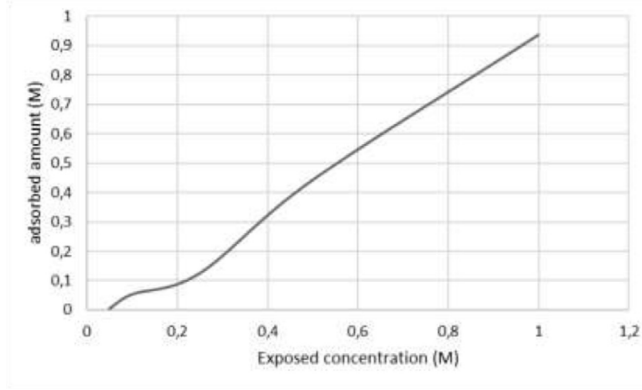

Fig. 1. Relationship between concentration of Sr to which seeds were exposed and amount of Sr adsorbed.

the counted seeds) after exposure to titanium for different lengths of time. Also, Çanli (2018) found them to be the abnormality with the highest frequency in lentil seeds after exposure to barium and vanadium, occurring in 36.4 and 26.7% of the counted seeds, respectively. In a similar study, chromosome adherence and bridge chromosomes were the most frequently observed abnormalities in cells of lettuce after exposure to an extract of *Macroptilium lathyroides* (Silva *et al.* 2018). In another study, Kiran & Şahin (2005) obtained similar results in testing the effects of lead exposure on *L. culinaris*: they found that concentration increase caused a decrease in cell division and several mitotic anomalies such as c mitosis, lagging chromosomes, multipolar anaphases and chromosome bridges. In our study, those abnormalities showed no regular distribution. After exposures to Sr for more than 8 hours, there was no seed production in the plant. This result showed that after 8 hours, disorder in mitotic cell division prevented the plant from producing any seeds.

Melnyk *et al.* (2019) determined that lentil can absorb more Sr than broccoli and spaghetti. They pointed out that lentil always absorbs 57% of Sr from the solution no matter what the concentration is. Just as for some plants like lettuce, which are accepted as good bioaccumulators of heavy metals (Smical *et al.* 2008; Antonkiewicz *et al.* 2016), *L. culinaris* was also found to be a good bioaccumulator of Sr in our study (absorbing at least 95%) unless the exposure time was more than 8 hours (when absorption was between 81 and 91%). In a similar study, Basu *et al.* (2017) investigated the ability of lentil husk to adsorb cadmium for purification of wastewater. They found that 1.0 g lentil husk can absorb 107.31 mg of cadmium.

As in the case of Melnyk's study (2019), in the present study we also found that Sr absorption reaches the highest level at a certain concentration and after a certain length of time: the seeds uptake Sr at first,

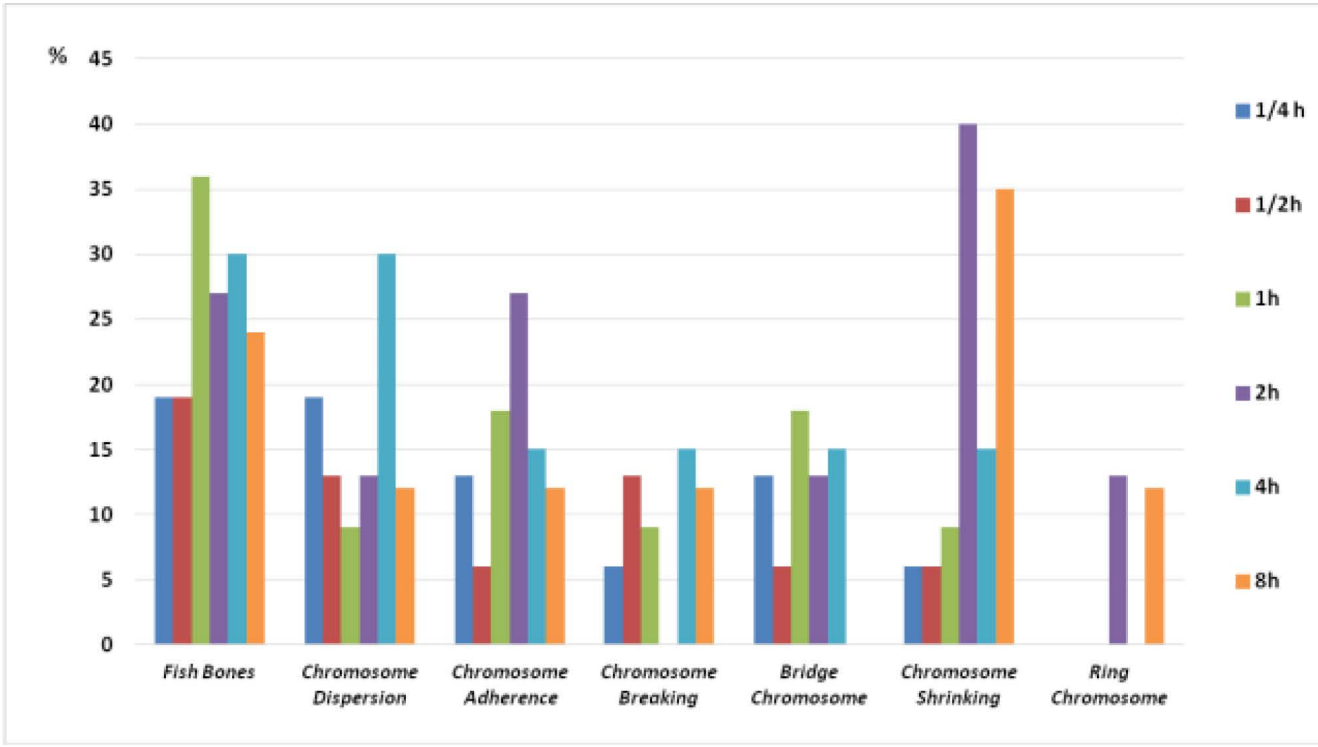

Fig. 2. Percentages of counted root tip cells of *L. culinaris* with different chromosome abnormalities as plotted against exposure time.

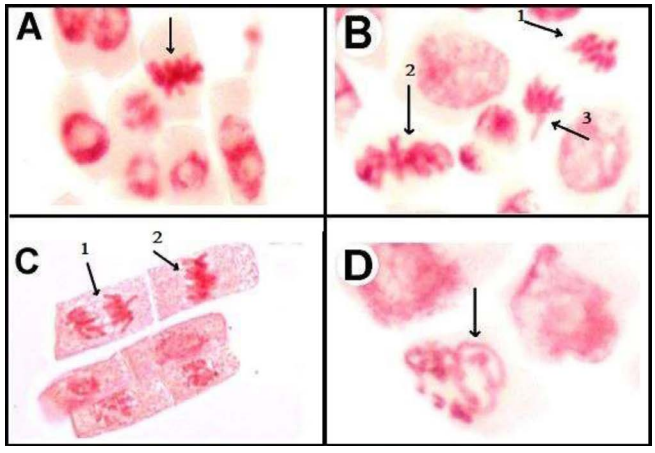

Fig. 3. Some investigated chromosome abnormalities (A: Chromosome shrinking; B: 1. chromosome breaking; 2, 3. chromosome dispersion; C: 1. bridge chromosome, 2. fish bone; D: ring chromosome).

then let the excess back into the solution. The amount of the chemical absorbed by the seeds is returned into the solution as time progresses. The fact is that some metal ions were transferred to the plants, while others were sent out from them, precisely as claimed by Yin-Xiao *et al.* (1986). The correlation between Sr concentration and the adsorbed amount of Sr exists because the chemical retention rate of seeds increased with increasing concentration.

CONCLUSION

Highlights of the study can be listed as below:

- There is no simple correlation between concentration, exposure time and the type of abnormalities.
- Every concentration of Sr to which seeds are exposed unquestionably creates at least one type of abnormality in the cells.
- Up to a certain time of exposure, lentil seeds can be used as a bioaccumulator for removal of Sr.

In the course of further study, lentil seeds can be applied for purposes of bioaccumulation to fields containing a high Sr concentration. Also, the impact of strontium on uptake of macroelements and macronutrients by lentil or other plants can be investigated in the future.

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REZIME

Botanica SERBICA

Koncentracija i vremenski zavisni efekti stroncijuma na *Lens culinaris* Medik.

Hakan Sepet i Murat Çanli

Ova studija istražuje kako joni stroncijuma (Sr) deluju na meristematske ćelije korena sočiva (*Lens culinaris*) sa promenljivim parametrima (vreme i koncentracija). Semena biljaka su bila izložena standardnim rastvorima stroncijuma u različitim vremenskim periodima (1/4, 1/2, 1, 2, 4, 8, 12, 16, 20, i 24 sati) u fiksnoj koncentraciji od 1.0 molL^{-1} (M) i jonima Sr u različitim koncentracijama (0.05, 0.1, 0.25, 0.5, i 1.0 M) u određenom vremenskom periodu (12 sati). Semena tretirana stroncijumom su proklijala i vrhovi korenčića su istraživani mikroskopom, sa ciljem razjašnjavanja hromozomskih abnormalnosti pri deobi ćelija. Istraživanja mikroskopom su pokazala anormalnosti u ćelijama klijanaca u smislu srastanja hromozoma, pucanja, hromozomskih mostova, disperzije hromozoma, njihovog skupljanja, riblje kosti i prstenastih hromozoma. Ove abnormalnosti su uočene nekoliko puta u svakom tretmanu zavisnom od različitih peroda i koncentracija. Adsorpcija i absorpcija Sr unutar semena sočiva su uočene spektroskopski. Spektroskopski su uočene i otklonjene i suvišne količine Sr. Zatim, statistička evaluacija rezultata je primenjena kako bi se uočila značajnost sličnosti i razlika. Rezultati ovih analiza su sa jedne strane ukazali na pozitivnu korelaciju sa koncentracijom kao parametrom, a sa druge, na negativnu korelaciju sa vremenom. Više od 90% Sr je uklonjeno iz rastvora tokom 12-časovne ekspozicije. Semena sočiva se mogu prihvatiti kao dobri bioakumulatori jona Sr jedino pri ekspoziciji u vremenskom periodu kraćem od 12 sati pri koncentraciji od 1 M Sr.

Ključne reči: abnormalnosti hromozoma, bioakumulator, sočivo, citogenetički efekti