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A new perspective to aberrations caused by barium and vanadium ions on *Lens culinaris* Medik

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ABSTRACT

This study investigates aberrations caused by barium and vanadium on meristematic cells of *Lens culinaris* Medik. Barium and vanadium ions at various concentrations (0.05 M, 0.1 M, 0.25 M, 0.5 M, and 1.0 M) were exposed to the seeds of the plant at fixed time interval (12 h). After seedlings, with a microscopic examination images were captured about the root tips. Those images showed that several abnormalities occurred on the plant such as chromosome breakings, chromosome dispersion, bridge chromosome, chromosome adherence, ring chromosome. Variety and number of abnormalities were counted and compared to each other statistically. The results show an increase in abnormalities caused by for both ions with increasing treatment time. Chromosome adherence and chromosome breaking have reverse relationship in which number of occurrence for one of them decreases with increase on other one. Fish bone and chromosome adherence have a positive relationship in which number of one increases with the raise in other's number. Exposed metals have caused formation of ligands with proteins which can prevent the persistence of metal ions in DNA protein cross-links that are involved in DNA formation process.

1. Introduction

Metals can move to plants from soils and accumulate inside roots, leaves and shoots (Maestri et al., 2010; Sepet et al., 2014). While some metals stimulate growth of the plants (Bhargava et al., 2012; Tangahu et al., 2011), several others make changes inside the plant chromosomes (Özdemir et al., 2015, 2012). Baranowska-Morek and Wierzbicka (2004) have studied on lead distribution inside plants, and found out a gathering on root tips of the plant. Janas et al. (2010) have found on their investigation that copper ions are accumulated in vacuoles and the cell wall of root cells of *Lens culinaris* (Medik.). Accumulation of metals occurs by binding with proteins and peptides. Six types of alteration have been mentioned as C-mitosis, stickiness, laggards, bridges, fragments and multipolarity (Kuchy et al., 2016; Özkul et al., 2016). Over certain amounts, transition metals can lead oxidation in tissues of plant (Schützendübel and Polle, 2002).

Among those metals, vanadium (V) is a side product of fossil fuels during energy production and several other industries like mining, metallurgical and galvanization (Tham et al., 2001). Oxidative forms of V have higher harmful effect than elemental form. Vanadium was recorded in various commercial nutritional supplements and multivitamins in amounts ranging from 0.0004 mg to 12.5 mg. At high concentrations, V ions shows very toxic effect especially in shoot of soybean, flax and oat plants (Warington, 1954). The plant species, the form of V and the soil type affect toxic effects of V in plants changing from 10 to 1300 mg/kg (World Health Organisation, 2000). Besides, V has also been reported as the inhibitor of seed germination and root elongation (Tham et al., 2001). While it reduces barley and tomato growth at 31 mg/kg-510 mg/kg (Larsson et al., 2013), at low concentrations, V ions were reported as not detrimental to several plants like bush bean plant and sweet basil (Akoumianaki-Ioannidou et al., 2016; Martin and Saco, 1995). Use of heavy oils, tar sands, and bitumen as combustion sources lead to increase V amount in atmosphere which causes kidney diseases in humans (Filler et al., 2017; Schlesinger et al., 2017).

As another metal, barium (Ba) has great impact for plant and animal growth because of its presence in the environment. Mainly, Ba concentration becomes toxic as a result of mining activities measured in the range of 0.13–29.2% (Lamb et al., 2013). In Canada, reported Ba amount in house dust varies from 190 to 1480 mg/kg (Canadian Council of Ministers of the Environment, 2013). High concentrations of Ba may only be found in soils and in food, like nuts and certain plants. Because of the widespread use of Ba in manufactured materials such as tiles, automobile clutch and brake linings, rubber, brick, paint, glass, and other human activities like traffic, this metal may have high concentrations in soils like ranging from 30.9 mg/kg to 1210 mg/kg (McBride et al., 2014). It finds its way in plants directly by foliage and via water and soil and eventually into animals when they consume Ba

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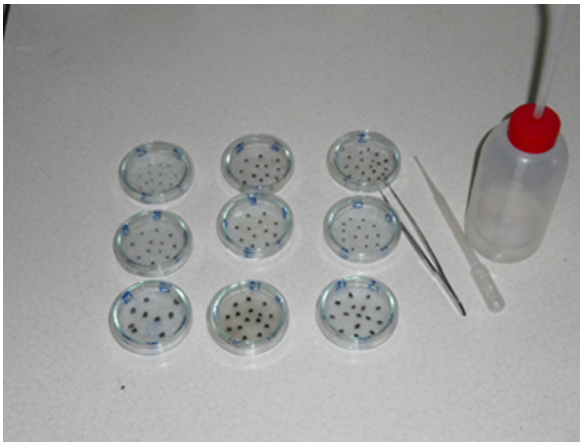

Fig. 1. Seeds left for seedling after exposure of metals.

enriched plants. The main source of barium accumulation in plants is from the soil. Ba amount in soils have been found from 15 mg/L to 3500 mg/L (Agency for toxic substances and disease registry, 2007). The most harmful effect of Ba is on plant growth. The critical threshold level of $BaCl_2$ for plant growth inhibition is quite variable. The inhibition of growth is an outcome of the damage to the physiological, cytological and biochemical processes.

Lentil experiences are being preferred in metal accumulation studies because it is showing off abiotic stresses, salinity, drought, and metal toxicity on its growth and yield (Talukdar, 2013). Lens culinaris has 14 (2 n) chromosomes. Chromose preparation is achieved from meristematic cells of roots of Lens culinaris as suggested in Plant Cytogenetics (Singh, 2003).

Even though metals have positive influence on plant growth at certain level, for higher concentrations, they can either stop or reduce the growth of seedlings. Because biological effects of V and Ba on plant cells show no clear results in previous research efforts (Abreu et al., 2012), this study offers a scenario to present how both metals are related to each other in terms of their cause on chromosomal change in root tips of Lens culinaris.

In this study, two questions were answered:

1. What is the concentration of V and Ba effecting plant growth?
2. How aberrations caused by V and Ba do relate or differ from each other?

2. Material and method

In this study, cytogenetic effects on the seeds of the plant were searched by treatment of metal ions at various concentrations (0.05 mol Ba/L, 0.1 mol Ba/L, 0.25 mol Ba/L, 0.5 mol Ba/L, and 1.0 mol Ba/L) of Ba and (0.05 mol V/L, 0.1 mol V/L, 0.25 mol V/L, 0.5 mol V/L, and 1.0 mol V/L) V at 12 h interval. For this purpose, water soluble solutions of Ba and V have been prepared. Ba was applied as $BaCl_2x2H_2O$ which was purchased from Merck, and as V source, V_2O_5 was used which was purchased from also Park Chemicals.

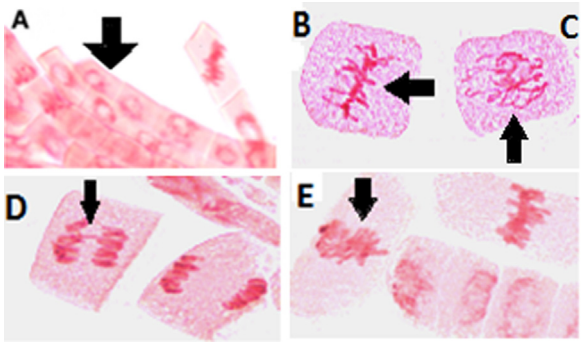

Fig. 2. Examples for Investigated chromosome abnormalities; (A) ring chromosome, (B) fish bones, (C) chromosome dispersions, (D) chromosome breaking, (E) chromosome adherence.

Table 2
Percentages for occurrences of abnormalities in root tip cells of *Lens culinaris* at different concentrations of Ba.

Investigated abnormality(%)								
Dose treated (mol/L)	**Treatment time (hour)**	**F.B.**	**C.D.**	**C.A.**	**C.B.**	**B.C.**	**C.S.**	**R.C.**
0.05	12	14.10	9.10	11.73	7.37	12.73	6.37	0
0.1	12	17.04	10.69	16.35	5.69	6.35	6.35	0
0.25	12	36.40	7.10	18.20	5.10	10.20	9.10	0
0.5	12	–	–	–	–	–	–	–
1.0	12	–	–	–	–	–	–	–

F.B.: Fish Bones, **C.D.**: Chromosome Dispersion, **C.A.**: Chromosome Adherence, **C.B.**: Chromosome Breaking, **B.C.**: Bridge Chromosome, **C.S.**: Chromosome Shrinking, **R.C.**: Ring Chromosome.

Table 3
Percentages for occurrences of abnormalities in root tip cells of *Lens culinaris* at different concentrations of V.

Investigated abnormality(%)								
Dose treated (mol/L)	**Treatment time (hour)**	**F.B.**	**C.D.**	**C.A.**	**C.B.**	**B.C.**	**C.S.**	**R.C.**
0.05	12	19.10	14.50	10.30	5.55	17.45	3.76	0
0.1	12	21.45	15.90	10.05	3.46	9.75	7.57	1.05
0.25	12	26.70	13.60	11.90	9.17	15.67	6.61	0.78
0.5	12	13.48	12.56	9.38	5.51	12.34	5.72	0.20
1.0	12	23.91	4.22	5.42	8.20	24.55	7.05	3.85

F.B.: Fish Bones, **C.D.**: Chromosome Dispersion, **C.A.**: Chromosome Adherence, **C.B.**: Chromosome Breaking, **B.C.**: Bridge Chromosome, **C.S.**: Chromosome Shrinking, **R.C.**: Ring Chromosome.

Before exposure of the metal solutions, plump, and undamaged lentil seeds with same size were exposed to sodium hypochlorite 10% for 10 min in order to prevent seed contamination. After exposure to metal ions for certain time periods, the seeds were washed by distilled

Table 1
The mitotic index of root tip cells of *Lens culinaris* at different concentrations of Ba and V.

Concentrations (mol/L)	Mitotic index ± *S.D. (for Ba)	Mitotic index ± *S.D. (for V)	Time (hour)
.05	**16.70 ± 6.75**	18.89 ± 7.21	**12**
0.1	**14.09 ± 3.90**	16.88 ± 4.40	**12**
0.25	**11.22 ± 3.62**	10.04 ± 3.11	**12**
0.5	–	7.63 ± 2.28	**12**
1.0	–	6.52 ± 2.22	**12**
Control Group	**18.12 ± 6.35**	19.34 ± 3.85	**12**

Table 4
Pearson coefficients and T-Test scores for obtained chromosome abnormalities on the root tip cells of L.*culinaris* after V and Ba exposure.

Variables	Value for V	Value for Ba	T-Test values
Fish bone and Chromosome shrinking	0,3469	0,9918	0,6325
Fish bone and Bridge Chromosome	0,3094	−0,0027	0,0132
Fish bone and Chromosome dispersion	0,7732	−0,8368	0,0254
Fish bone and Chromosome adherence	0,5499	0,7995	0,0337
Fish bone and Chromosome breaking	−0,0730	−0,7823	0,0112
Chromosome dispersion and chromosome adherence	−0,0290	−0,3402	0,9405
Chromosome dispersion and chromosome breaking	0,4499	0,3136	0,0112
Chromosome dispersion and bridge chromosome	0,2332	−0,5452	0,0132
Chromosome dispersion and chromosome shrinking	−0,1156	−0,8998	0,6325
Chromosome dispersion and ring chromosome	−0,3004	Not calculated	0,0044
Chromosome adherence and chromosome breaking	−0,8399	−0,9996	0,3408
Chromosome adherence and chromosome shrinking	0,5582	0,7163	0,4062
Chromosome adherence and bridge chromosome	0,4564	Not calculated	0,2524
Chromosome breaking and bridge chromosome	−0,1834	0,6250	0,7289
Chromosome breaking and chromosome shrinking	−0,6202	−0,6965	0,2617
Bridge chromosome and chromosome shrinking	−0,4724	0,1249	0,3286

Table 5
Pearson coefficients for correlation among abnormalities and concentrations.

Pearson coefficients	Variables (comparing two metals)
0,6505	Fish bone
0,6463	Chromosome dispersion
0,5458	Chromosome adherence
−0,0456	Chromosome breaking
−0,6111	Bridge Chromosome
0,8728	Chromosome shrinking
−0,3981	0,05 M
0,5156	0,1 M
0,4019	0,25 M

water, and left for germination in petri dishes at 20–25 °C (Fig. 1). Seedlings were fixed by cutting from 1.5 to 2.0 cm end of the root tips, and they are placed directly into fixative solution (in the ratio of 3:1, 100% ethyl alcohol: glacial acetic acid).

The root tips of sprouted seeds were used for microscopic examination. Stock root tips of the plant were colored by Feulgen method to get more obvious images in microscopic examination. It is one of the most reliable and most specific technique for DNA (Akhtar and Chantziantoniou, 1998). In this method, the reaction is based on the release of active aldehyde groups by cleavage of the purine-deoxyribose bonds by acid hydrolysis. Aldehydes change the color of Schiff reagent in order to give a violet-violet color chromatin. This reaction is analogous to the periodic acid- Schiff reaction (Mello and Vidal, 2017).

Cytogenetic examination has focused on the cells in homologous areas. The mitotic cells was detected and counted. Any noticed chromosomal aberrations were photographed with Olympus Stream microscope. The images from different preparations at different Ba and V concentrations on the plant seeds were compared to each other, and the results were analyzed. For all concentrations, 10 lentil seeds were used, and the experiments were repeated four times.

3. Results

Exposure of Ba and V standard solutions to the seeds of Lens culinaris at different concentrations has caused an increase of mitotic cell division in their seedlings. Mitotic index (MI) showed that the cell division in seedlings was observed at lower level in all treatment time than control group (Table 1). Table 1 shows that with increase in V concentration mitotic cell division gets lower. For Ba exposure, at 0.5 and 1.0 M concentrations there are no seedling occurred. Comparison of both ions shows that mitotic index for V has higher values than mitotic index for Ba. Beside changing MI values, microscopic examination showed that several abnormalities occurred on the plant such as chromosome breakings, chromosome dispersion, bridge chromosome, chromosome adherence, ring chromosome (Fig. 2). In Tables 2, 3, the highest abnormality percentage for both Ba and V is in the fishbone. The lowest ratios are for ring chromosome type of abnormalities.

Correlations between variables of abnormality types were statistically calculated (Tables 4, 5). After Ba exposure, pearson coefficient of fish bone and chromosome dispersion was found as −0.8368. This coefficient which indicates the relationship between those variables was 0.1248 for bridge chromosome and chromosome shrinking. While chromosome dispersion and chromosome shrinking had −0.8998 for pearson coefficient, fish bone and chromosome shrinking had higher value of pearson coefficient as 0.9918. The lowest value for correlation was found for fish bone and bridge chromosome as −0.0027. On the other hand, the highest value for correlation was calculated for chromosome adherence and chromosome breaking as −0.9996. For V exposure, the highest value was noted as −0.8399 between chromosome adherence and chromosome breaking. The lowest pearson coefficient was determined as −0.0290 between chromosome dispersion and chromosome adherence.

For *t*-test scores of abnormalities, the lowest values were found for chromosome shrinking-ring chromosome, chromosome adherence-ring chromosome, chromosome dispersion-ring chromosome, chromosome dispersion-bridge chromosome, and chromosome dispersion-chromosome breaking as 0.0151, 0.0288, 0.0044, 0.0132, and 0.0112 respectively (Table 4). These numbers show that there is a statistically significant difference between metals in terms of mentioned aberrations.

Comparison of both metals showed that they have the lowest correlation at chromosome breaking and the highest correlation at chromosome shrinking (Table 5). Ba and V exposure on the plant chromosomes have created the same changes for several chromosomal aberrations (for fishbone, chromosome dispersion, chromosome adherence, and chromosome shrinking as 0.6505, 0.6463, 0.5458, and 0.8728, respectively). This means that those aberrations mentioned above happen in similar ratios for both metals. The lowest correlation has occurred on chromosome breaking between metals (−0.0456). In another words, while one metal caused an increase in chromosome breaking, the other one had a decrease in the number of chromosomes showing chromosome breaking. When concentrations considered as variables, the values of pearson coefficient have changed between −0.3981 and 0.5156. Because of no seedlings happened for Ba exposed seeds over 0.5 mol/L, there was no correlation determined for 0.5 mol/L and 1.0 mol/L.

4. Discussion

Both metal solutions have caused to several abnormalities as fish bones chromosome adherence, chromosome dispersions, chromosomal

adherence, bridge chromosome, chromosome breaking, chromosome shrinking, ring chromosome. Mostly, those effects occur because of covalent and noncovalent binding modes of metal complexes within DNA. Metals make the connection to DNA compounds interfering with hydrogen bonds which hold DNA together.

Fish bone and chromosome shrinking had the highest occurrence frequency. There is a good correlation between two metals showed that chromosome adherence, chromosome shrinking, chromosome dispersion and fish bone occurred in the same raising ratio in root tips of lentil seedlings for both metals. In a similar study, after copper chloride exposure on root tip cells of *Vicia hirsuta* (L), and the most observed abnormalities were noticed as chromosome adherence and bridge chromosome (İnceer and Beyazoğlu, 2000). In another study, Kıran and Sahin (2005) called that increase of the lead concentration on the root tip cells of lentil (*Lens culinaris* Medik.) has caused several mitotic anomalies such as c mitosis, lagging chromosomes, multipolar anaphases and chromosome bridges.

This study also revealed out that at certain contact time with metal exposure, the mitotic cell division of the root tips of *Lens culinaris* reduced. The changes in MI of *Lens culinaris* cells are indicators of cytogenetic activity of Ba and V. The results showed that higher concentrations were able to inhibit significantly cell division (Li et al., 2017; Nefic et al., 2013). Frequency of those abnormalities did not follow any order. While there was no seedling happened for Ba exposed seeds of the plant at the concentrations over 0.25 mol/L, V exposed plant seeds had continued cell division during all concentrations. However, some aberrations happened in both metal exposures which show both metals act in same way on the plant chromosomes. V exposure has also created ring chromosomes in lentil seedlings which is different than Ba exposure.

In this study, exposed metals have caused formation of ligands with proteins inside plants' DNA. Potential ligands with transition and heavy metals can prevent the persistence of metal ions in DNA protein crosslinks that are involved in DNA formation process (inversions, translocations, centric split and fusion, duplications, and deletions) (Bhargava et al., 2012; Maestri et al., 2010; Muszyńska and Hanus-Fajerska, 2015; Seth et al., 2008; Tangahu et al., 2011). In this case, just like *Drosophila buzzatii*, lentil chromosomes showed polymorphic inversions by recombination of the breakpoints containing large insertions by transposable elements (Schubert et al., 1994; Cáceres et al., 1999). Several chromosomal rearrangements and polyploidy took place in lentil mitotic cells in terms of choromosomal changes (Wu and Tanksley, 2010; Schubert and Lysak, 2011; Dodsworth et al., 2016; Moraes et al., 2017). While some chromosomes lose their parts and sizes (ring chromosome) (Schubert and Lysak, 2011), some others have duplication or inversion on them (Badaeva et al., 2007; Idziak et al., 2014; Degrandi et al., 2017).

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