Turk J Biol
32 (2008) 73-78

Lead-Induced Cytotoxicity and Mutagenicity in Grass Pea

Girjesh KUMAR, Ritambhara TRIPATHI
Plant Genetics Laboratory, Department of Botany, University of Allahabad, Allahabad, 211002 - INDIA

Received: 23.05.2007

Abstract: Chromosomal variation is an important tool in genetic analysis. The objectives of the present study were to determine the mutagenic potential of lead (Pb) on the meiotic cells of grass pea (*Lathyrus sativus*) and to determine the maximum concentration of lead nitrate that induces maximum genetic variability in grass pea. The different concentrations of lead nitrate used were 25, 50, 100, 200, and 300 ppm. The percentage of abnormalities increased with the lead nitrate concentration. Different types of chromosomal abnormalities were observed, such as condensed bivalents, secondary association, laggards, bridges, cytomixis, stickiness, etc.

Key Words: Chromosomal variation, lead (Pb), mutagenic potentialities, *Lathyrus sativus*, cytomixis, stickiness

Burçaklarda Kurşun ile Oluşturulan Sitotoksiklik ve Mutasyon Oluşumu

Özet: Genetik analiz işlemlerinde kromozomal varyasyonlar önemli rol oynarlar. Bu çalışmanın amacı burçak bitkisinin (*Lathyrus sativus*) mayotik hücreleri üzerinde kurşunun mutajenik potansiyelini belirlemek ve burçak bitkisinde maksimum genetik varyasyon orataya çıkarabilecek kurşun nitrat konsantrasyonun ortaya çıkarmaktır. 25, 50, 100, 200 ve 300 ppm konsantrasyonlarında kurşun nitrat kullanılmıştır. Doz ile orantılı olarak anormallik yüzdeleri artmıştır Yoğun bivalenler, ikincil yapılar, geri kalmış kromozomlar, köprüler, sitomiksis, yapışkanlık şekinde kromozom anarmallikleri gözlenmiştir

Anahtar Sözcükler: Kromozomal varyasyon, kurşun (Pb), mutagenik potensiel, *Lathyrus sativus*

Introduction

Presently, parallel to the rapid growth in industrialization environmental pollution is also increasing. Heavy metals like Cd, Cu, Fe, Hg, and Pb contribute significantly to the growing pollution problem. Environmental contamination and exposure to heavy metals is a serious problem worldwide. It is well known that heavy metals are among the most toxic and environmentally dangerous pollutants.

A number of authors have discussed this issue and determined the mutual relationships of heavy metals in natural and industrial environments, the increased frequency of chromosome mutations, and the cancerous processes in organisms (1-3). Pollutants of this kind at ppm level usually cause anomalies in mitotic division. Moreover, at higher concentrations, heavy metal ions react to form toxic compounds in cells (4). Chromosomal rearrangements are one of the most frequently produced classes of mutation that result from the action of both physical and chemical mutagenic agents (5).

Among the heavy metals, the use of lead (Pb) during the last 50 years has caused widespread environmental contamination. As lead causes a variety of toxic effects, the fate of lead in the environment is of great concern.

The present study, therefore, aimed to evaluate the effects of lead on the meiotic cells of grass pea (*Lathyrus sativus*). Grass pea, furthermore, has a great agronomic potential as a grain and forage legume. Since it is considered one of the most promising sources of protein for the vast and expanding populations of Asia and Africa, along with its stress tolerance adaptability, grass pea may provide a good model for cytogenetic studies and for producing new offspring with genetic adaptations to withstand heavy metal stresses.

At the same time, the number of studies on the cytotoxic and mutagenic effects of heavy metals on grass

pea is rather limited. The present investigation, therefore, has been carried out to evaluate the cytotoxic effects of lead on the meiosis of grass pea and to determine the maximum concentration of lead nitrate that induces maximum genetic variability.

Materials and Methods

The seeds of *Lathyrus sativus* L. var. Pusa-24 were procured from the National Bureau of Plant Genetic Resources, New Delhi. The seeds were presoaked in distilled water for 12 h. In all, 5 different concentrations of $PbNO_3$ (25, 50, 100, 200, and 300 ppm) were prepared in distilled water. The soil in the experimental pots was amended with these concentrations separately and the seeds were sown in the experimental pots along with the control set.

For meiotic studies, flower buds were fixed in ethanol:acetic acid (3:1) for 24 h. and transferred to 70% alcohol. Temporary slides were prepared by the squash technique and stained with 1% acetocarmine (6). In all, 10 slides were examined for each concentration of $PbNO_3$ to evaluate the standard error value. To ascertain viability, the size of M_1 generation seeds was taken into consideration.

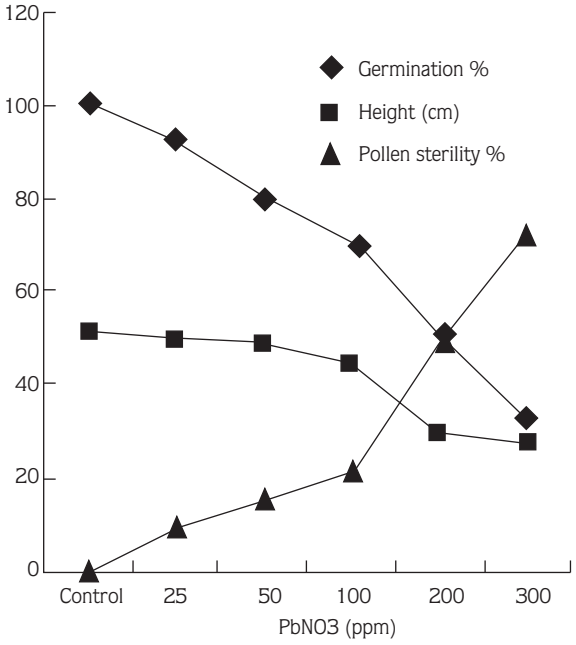


Figure 1. Graphical representation of seed germination (%), plant height (cm), and pollen sterility (%) in grass pea treated with different concentrations of $PbNO_3$.

Observations

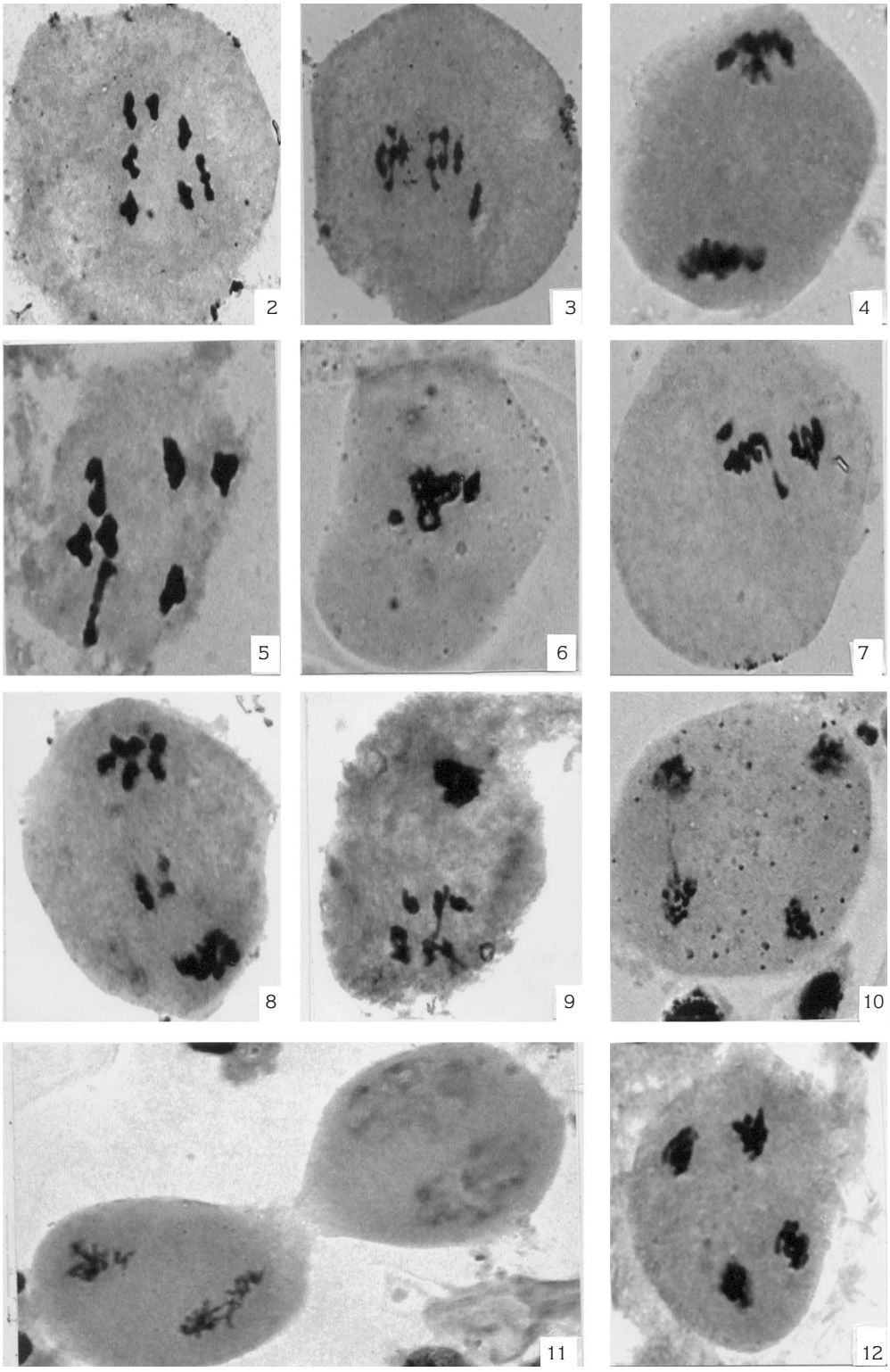
The results are based on the observation of morphological and cytological parameters. Approximately 100% of the untreated seeds germinated, while germination among the treated seeds decreased with increasing concentrations of $PbNO_3$ (93.3%-33.5%) (Figure 1). The height of M_1 generation plants was inversely proportional to the concentration of $PbNO_3$ (Figure 1); however, a lower percentage of pollen sterility was observed at 50 and 100 ppm concentrations of $PbNO_3$, as compared to higher concentrations, which resulted in a tremendous increase in sterility, i.e. up to 72.4% (Figure 1).

Conventional cytological analysis was conducted and the presence of 7 bivalents at diakinesis (Figure 2), metaphase I (Figure 3), and regular separation of 7:7 at anaphase I (Figure 4) was observed in the control plants. However, cytological analysis also documented numerous anomalies in the pollen mother cells (PMCs) of $PbNO_3$-treated plants (Table). An interesting abnormality recorded in the diakinesis stage of treated plants was the presence of large and condensed bivalents with disrupted PMCs at higher concentrations (Figure 5). Secondary association at metaphase I plate (Figure 6) was frequently observed at all the concentrations.

Proceeding to further stages of division, several other abnormalities were observed, such as precocious movement at metaphase I (Figure 7), laggards at anaphase I (Figure 8), stickiness at anaphase I (Figure 9), and bridge at anaphase II (Figure 10). One of the most interesting abnormalities was cytoplasmic connection between the PMCs at different stages of division (Figure 11) and stickiness at anaphase II (Figure 12). The maximum percentage of cytomixis (15.3%) was observed at the 100-ppm concentration of $PbNO_3$ (Table). Stickiness was observed to increase drastically at higher concentrations of $PbNO_3$ (Table).

Discussion

The results of the present study demonstrate that heavy metal (Pb) stress had a significant effect on the phenotype and genetic structure of M_1 generation plants. The observed chromosomal aberrations, on the other hand, represent the direct effect of pollution on the genetic material.



Meiotic configurations in control set (1-3) Figure 2. Diakinesis (n = 7). Figure 3. Metaphase I (n = 7). Figure 4. Anaphase I (7:7 separation). Meiotic configurations in lead treated set (4-11) Figure 5. Dense and enlarged bivalents with distorted PMC. Figure 6. Secondary association at metaphase I. Figure 7. Precocious movement at metaphase I. Figure 8. Laggards at anaphase I. Figure 9. Stickiness on pole at anaphase I. Figure 10. Single bridge at anaphase II. Figure 11. Cytomixis between PMCs of 2 different stages. Figure 12. Stickiness at anaphase II.

Table. Percentage of various abnormalities in Pb-treated grass pea.

Dose (ppm)	Total PMCs observed	Types of Abnormalities (%)						
		Metaphase I/II Mean ± SE			Anaphase I/II Mean ± SE			
		Sec. assoc. Mean ± SE	Pm Mean ± SE	Cy Mean ± SE	Lg Mean ± SE	Bg Mean ± SE	St Mean ± SE	Tab Mean ± SE
Control	110	-	-	-	-	-	-	-
25	108	1.2 ± 0.34	2.6 ± 1.76	2.8 ± 0.29	1.4 ± 0.07	2.4 ± 0.76	1.8 ± 0.79	12.2 ± 0.43
50	115	2.5 ± 1.87	3.8 ± 1.93	4.6 ± 2.01	2.8 ± 0.99	4.1 ± 1.98	2.2 ± 1.07	20.2 ± 1.67
100	108	3.6 ± 2.01	4.2 ± 2.04	15.3 ± 3.99	3.4 ± 1.98	4.8 ± 2.01	4.6 ± 2.03	35.9 ± 2.06
200	120	6.8 ± 2.65	6.1 ± 2.07	10.2 ± 2.76	6.2 ± 2.07	10.2 ± 2.35	14.8 ± 3.17	54.3 ± 2.41
300	118	10.2 ± 2.79	9.4 ± 2.03	12.4 ± 2.89	11.4 ± 1.97	18.4 ± 2.95	22.8 ± 2.56	84.6 ± 2.98

Sec. assoc.: secondary association; Pm: precocious movement; Cy: cytomixis; Lg: laggard; Bg: bridge; St: stickiness; Tab: total abnormality.
All values are expressed as mean ± SE.

Cytological investigations clearly revealed that the level of chromosomal aberration gradually increased along with increasing concentrations of $PbNO_3$. In addition, the tolerance of the plant to environmental stress was also marked, up to the 100-ppm concentration. According to Liu et al. (7) and Ishido et al. (8), a decrease in the frequency of cell division represents an example of the inhibitory effect of heavy metal ions. Their studies also indicate that the application of heavy metals to seeds at levels of 10-200 ppm inhibits the growth of plumula and radicles. Other characteristics that were negatively affected include germination percentage, germination index, root-shoot length, and dry matter content (9). Some studies claim that heavy metals, particularly Pb and Cd, in exhaust gases, engine oils, and vehicle tires, inhibit pollen germination and tube growth in plants (10). There are also findings indicating that heavy metals cause cytogenetic anomalies in plants, such as inhibited mitotic division, decreased mitotic index, and chromosomal anomalies (11).

The activity of heavy metal ions (Zn^{++} ions) that induce chromosomal abnormalities in plants has been demonstrated by El-Ghamery et al. (12), and Grant and Owens (13). In the present study, no significant differences were found between the control and the lower $PbNO_3$ concentrations (25 and 50 ppm) applied. This may have been due to less toxicity associated with lower concentrations of the heavy metal under consideration.

The main cause of germination reduction is attributed to the occurrence of seeds without completely developed embryos (14). The analysis of M_1 plants in the present study allowed concluding that plant height was reduced, as compared to the control. This reduction can be attributed to chromosomal damage. Dimitrova (15) reported that high concentrations of Pb, Zn, Cd, and Cu suppressed the growth of vegetative organs. Dimitrova and Ivanova (16) also reported that increased levels of heavy metals in soil decreased not only the growth of vegetative organs, but the rate of their cell division, inducing chromosomal aberrations in *Linum usitatissimum*.

In our opinion, the condensed and enlarged bivalents observed at the higher $PbNO_3$ concentrations (200 and 300 ppm) may have been due to the effect of intrachromosomal mutation, which might have led to morphological alterations. According to Nicklas and Ward (17), non-oriented bivalents may be related to impaired attachment of kinetochores to spindle fibers. Pagliarini (18) reported that laggards may result from late chiasma terminalization. Similar results were obtained by Bipasha and Shella (19), and Srivastava and Srivastava (20) after administering heavy metals.

According to Utsunomiya et al. (21), ascending chromosomes are the result of precocious migration. Laggards and non-oriented chromosomes may produce micronuclei if they fail to reach the poles in time to be included in the main telophase nucleus (21). The anaphasic bridges obtained might be due to structural changes to deficiency and translocation type, some of them surviving until the late telophase, indicative of their stability.

Although cytomixis has been reported in several plant species, its origin is not clearly understood. Among the causal factors suggested for cytomixis, one of the probable factors may be chemical mutagens (22,23).

Chromosomal stickiness is characterized by clustering during any phase of the cell cycle. Several agents have been reported to cause chromosome stickiness, including chemicals present in soil (24). Boyadjiev (25) reported that Pb has embryotoxic and gonadotoxic effects. Chang et al. (26), using the *Allium cepa* test, proved the genotoxic effects of Pb-contaminated soil.

Earlier reports have shown that Pb causes chromosomal aberrations and spindle damage (19), and it is known to concentrate in cell nuclei and higher doses and cause cytotoxicity, though the mutagenicity of lead remains questionable. During the present study a variety of chromosomal aberrations were observed, which clearly indicate that the damage occurred at the chromosomal level and would persist in later generations. Hence, the higher concentrations of $PbNO_3$ were cytotoxic as well as mutagenic.

Although many authors reported chromosomal abnormalities induced in plants, few have emphasized the production of viable plants carrying chromosomal abnormalities. The present study determined that grass pea can tolerate Pb toxicity up to 100 ppm without losing viability, but that Pb was genotoxic and mutagenic at the concentrations above 100 ppm. The study resulted in the production of chromosome-altered grass pea plants that maintained vigor and fertility, which facilitates the perpetuation of the induced chromosomal variations up to the tolerable Pb levels.

Acknowledgement

The authors are grateful to N.B.P.G.R., New Delhi for supplying *Lathyrus sativus* seeds and to the members of the plant genetics laboratory.

Corresponding author:
Girjesh KUMAR
Plant Genetics Laboratory,
Department of Botany,
University of Allahabad,
Allahabad-211002, U.P. - INDIA
E-mail: kumar_girjesh@yahoo.com

References

1. Tzonevski D, Sapundzhiev K, Vodenicharov E. Studying the content of lead and cadmium in the blood of children up to 15 years old from the region of Non-ferrous metal works near Plovdiv Higiena Zdraveopazvane 2/3: 20-22, 1998.
2. Bruning T, Chronz C. Occurrence of urinary tract tumours in miners highly exposed to Dinitrotoluene. YOEM. 3: 144-149, 1999.
3. Chernozemski I, Shipkov T. Oncology. SIELA-SOFT. Sofia, 2001.
4. Nies DH. Microbial heavy metal resistance. Appl. Microbial Biotechnol. 51: 730-750, 1999.
5. Gecheff KI. Production and identification of new structural chromosome mutations in barley (*Hordeum vulgare*). Theor. Appl. Genet. 92: 777-781, 1996.
6. Belling J. The iron-aceto-carmine method of fixing and staining. Bil. Bull. 150: 160-162, 1926.
7. Liu DH, Liu WS, Jiang W et al. Evaluation of metal ion toxicity on root tip cells by *Allium* test. Israel J. Plant Sci. 43: 125-133, 1995.
8. Ishido M, Kunimoto M. Regulation of cell fate by Cadmium and Zinc. J. Health Sci. 47: 9-13, 2000.
9. Mishra A, Choudhary MA. Monitoring of phytotoxicity of lead and mercury from germination and early seedling growth indices in two rice cultivars. Kluwer Academic Publishers: Kluwer online abstract, volume: 114, issue: 3/4: pp: 339-346, 1999.
10. Yücel E. Asya servi kavağı kullanılarak Kütahya ilinde trafik kökenli Pb, Cd ve Zn kirliliğinin araştırılması. Tr. J. of Botany, 20: 113-116, 1996.
11. İnceer H, Beyazoğlu, O. Bakır Klorur'ün *Vicia hirsuta* (L) S.F. Gray kök ucu hücreleri üzerine sitogenetik etkileri. Turk. J. of Biol. 24: 553-559, 2000.
12. El-Ghamery AA, El-Kholy MA, Abov EL-Yousser MA. Evaluation of cytological effects of Zn^{++} in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. Mutat. Res. 537: 29-41, 2003.
13. Grant WF, Owens ET. Chromosome aberration assays in *Pisum* for the study of environmental mutagens. Review. Mutat. Res. 488: 93-118, 2001.
14. Falque M. Pod and seed development and phenotype of M1 plants after pollination and fertilization with irradiated pollen in cacao (*Thoebroma cacao*). Euphytica 75: 19-25, 1994.

15. Dimitrova I. Studies of grass plants under conditions of industrial pollution with heavy metals and sulphur dioxide. Author's summary of Ph.D. Dissertation, "P. Hilendarski". Plovdiv University, 1993.

16. I, Ivanova E. Effect of heavy metal soil pollution on some morphological and cytogenetical characteristics of flax. J of Balkan Ecology 6: 212-218, 2003.

17. Nicklas RB, Ward SC. Elements of error correction in mitosis, microtubule capture, release and tension. J. Cell Biol. 126: 1241-1253, 1994.

18. Pagliarini MS. Meiotic behaviour and pollen fertility in *Aptenia cordifolia*. Aizoaceae. Caryologia 43: 157-162, 1990.

19. Bipasha C, Shella S. Toxicity of some heavy metals in vivo and in vitro in *Helianthus annuus*. Mutat. Res. 283: 287-294, 1992.

20. Srivastava R, Srivastava, GK. Meiotic abnormalities and Tetraploidy (by Lead nitrate) induced by heavy metals (Pb, Cu, Hg, Zn) on *Helianthus annuus* L. Cytologia 69 (2): 119-124, 2004.

21. Utsunomiya KS, Bione NCP, Pagliarini MS. How many different kinds of abnormalities could be found in unique endogamous maize plant? Cytologia 67: 169-176, 2002.

22. Kumar G, Srivastava U. Cytomictic variations in Isabgol (*Plantago ovata*). Nucleus 44: 180-182, 2001.

23. Kumar G, Sharma V. Induced cytomixis in chickpea (*Cicer arietinum*) Nucleus 45: 24-26, 2002.

24. Caetano-Pereira CM, Pagliarini MS, Brasil, et al. Influence of aluminium in causing chromosome stickiness in maize microsporocytes. Maydica 40: 325-330, 1995a.

25. Boyadjiev V, Alexieva TsV, Zaprjanov Zk, et al. Heavy metal poisonings. Medcinai Fizkultura. Sofia, 1990.

26. Chang L, Meir J, Smith M. Application of plant and earthworm bioassays to evaluate remediation of a lead contaminated soil. Arch. Environ. Contam. Toxicol. 32: 166-171, 1997.