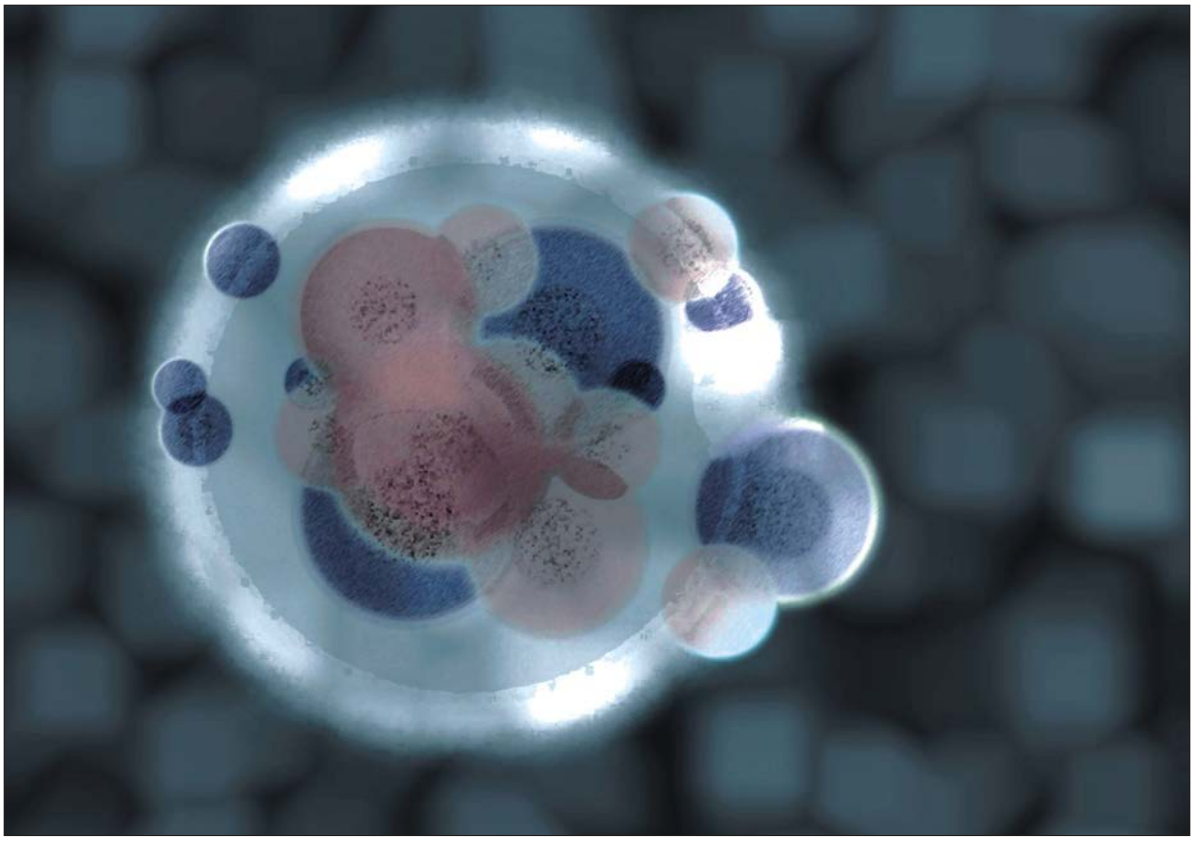

Journal of Biological Sciences

ISSN 1727-3048

science alert

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2018.208.215

Research Article
Evaluation of Genotoxic Potential of Heavy Metal in a Proteinaceous Crop (*Lens culinaris* Medik) in Aspect to Cyto-morphological Parameters

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Abstract

Background and Objective: Induce mutagenesis are known to induce variation in germplasm of plants for crop improvement programmes and as alternative to hybridization and recombination in plant breeding. The present study aimed to determine effects of lead (Pb) on cyto-morphological and growth parameters of lentil in M_1 generation. **Materials and Methods:** Germplasm of lentil were treated with different doses (5, 10, 15, 20 and 25 ppm) of lead nitrate. Different traits of control and mutagenized population was screened time to time and statistical analysis such as mean, Standard Deviation (SD), Coefficient of Variation (CV%) and Least Significant Difference (LSD at 1 and 5% level) were done. **Results:** Different qualitative and quantitative traits of control and treated population of M_1 generation were observed. Morphological parameters such as plant height, number of branches per plant, pollen fertility and yield was significantly reduced and showed variation in plant at the seedling stage as well as at mature stages. In addition to this, lead affected the pairing of homologous chromosomes with increasing concentration of lead nitrate percentage of chromosomal abnormalities also increase. Various types of meiotic lesion were recorded such as univalents, multivalent, laggards, bridges, stickiness, stray bivalent, disturbed polarity, precocious separation etc. **Conclusion:** Lower concentration of lead did not significantly affect the cytological and morphological parameters of lentil whereas higher concentrations of lead nitrate were found to be more genotoxic and mutagenic.

Key words: Induced mutagenesis, cyto-morphology, lead nitrate, lentil, genotoxic

Received: February 23, 2018 Accepted: April 06, 2018 Published: June 15, 2018

Citation: Durre Shahwar, M.Y.K. Ansari and Sana Choudhary, 2018. Evaluation of genotoxic potential of heavy metal in a proteinaceous crop (*Lens culinaris* Medik) in aspect to cyto-morphological parameters. J. Biol. Sci., 18: 208-215.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Heavy metal contamination in environment is due to effluents of industries, mining, agricultural chemicals ignition by product and fuel etc[1]. From past few decades, its concentration increases day by day because of production of industrial effluents such as various types of organic and inorganic pollutants, radioactive trace elements and microorganism which are becoming soil contaminant. Among these contaminants, heavy metals such as Pb, Cd, Hg, Ni are major affecting metal in all over the world and exhibit toxicity for plant, animal and human beings. According to Bruning *et al.*[2], a relationship was found between heavy metal of natural and industrial environment and an enhance frequency of chromosomal variation and cancerous process in organism. It was recorded that Pb has capacity to produce micronucleus formations[3-4], DNA damage[5], perturbation in meiosis[6] and changes in membrane permeability[1]. Recently, it has been investigated by researchers that DNA breaks are caused by Pb in tobacco[5] and in lupin[7]. According to Patra *et al.*[4], Pb reduce the mitotic stage and extend the interphase and thus prolonged the cell cycle of cells of *Vicia faba* roots.

Lens culinaris Medik, belongs to family Fabaceae 2n = 14, proteinaceous crop consumed as food to fulfill protein requirement of body. Instead of protein, lentil is source of carbohydrates, different type of vitamins, macro and micro mineral nutrients, fat and non nutritional bioactive phytochemical. Lentil also has several biological activities such as antioxidant, anticarcinogenic, antidiabetic, blood pressure lowering effect, antibiotic, anti-inflammatory etc[8,9].

The aim of the present study was to investigate the influence of Pb concentrations on somatic as well as reproductive cells of lentil, after all most conspicuous effect of heavy metals on plant development is growth inhibition which is correlated with cell division.

MATERIALS AND METHODS

Germplasm of *Lens culinaris* were collected from IARI, New Delhi, India. Healthy, fresh and uniform seeds of lentil were presoaked in distilled water for overnight and mutagenized with different doses (5, 10, 15, 20 and 25 ppm) of lead nitrate ($PbNO_3)_2$ solutions prepared in phosphate buffer (pH 4) for 12 h. One set of seed was soaked in distilled water to act as a control. Treated seed with mutagen were washed in running tap water to clean the adhered particle of mutagen particle from seed coat and were sown in the rabi season of the year 2015-2016 (Nov-April) along with respective control to grow M_1 generation plants.

Different quantitative and qualitative characters were observed in control as well as treated population. For meiotic experiment, young flower buds were selected from random plants of each concentration of mutagenized population as well as from untreated plants and fixed in carnoy's fluid (absolute alcohol, chloroform and acetic acid 6:3:1) for 24 h and stored in 70% alcohol. Anthers of collected flower bud were squashed in 1.0% propionic carmine and prepared permanent slide by using NBA-GAA series for pollen mother cells (PMCs) and pollen fertility analysis. Photographs were captured from both temporary as well as permanent slides.

Statistical analysis: Four replicates of each treatment were arranged. Statistical studies of data were performed with SPPS 17.0 for Window (SPSS, Chicago, IL, USA). Analysis of variance (ANOVA) was done to determine Least Significant Difference (LSD) between treatments; means were set at 5 and 1% significance levels ($p<0.05, 0.01$).

RESULTS

Observed data for cyto-morphological parameters in *Lens culinaris* Medik are presented in Table 1-3 and Fig. 1-2. Highest germination percentage and plant

Table 1: Germination, plant survival and pollen fertility in $Pb(NO_3)_2$ treated *Lens culinaris* Medik (M_1 Generation)

Concentration (ppm)	Germination (%)	Inhibition in seed germination (%)	Plant survival (%)	Lethality (%)	Variation frequency (%)	Pollen fertility (%)	Pollen sterility (%)
Control	95.00	0.00	96.84	0.00	0.00	95.60	0.00
$Pb(NO_3)_2$							
5	77.00	18.94	81.81	15.52	19.04	80.00	16.31
10	71.00	25.26	80.28	17.10	26.31	76.57	19.90
15	64.00	32.63	76.56	20.94	32.65	70.51	26.24
20	59.00	37.89	74.57	22.99	40.90	68.62	28.22
25	47.00	50.52	70.21	27.49	45.45	65.45	31.53

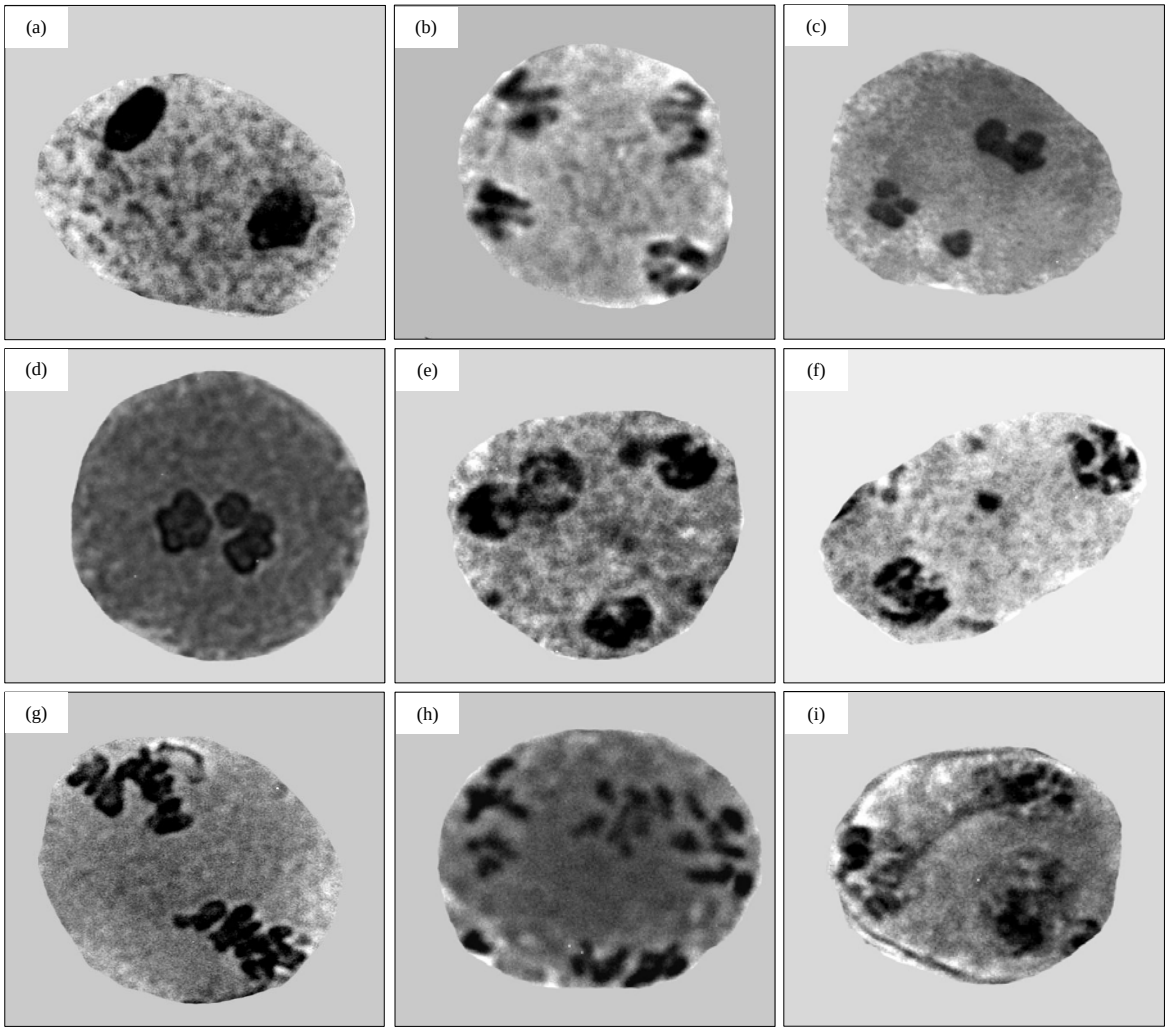

Fig. 1(a-i): PMC showing (a) Telophase I (control), (b) Anaphase II (control), (c) Laggard at anaphase I, (d) Stickiness at metaphase I, (e) Disturb polarity with 4 laggard at telophase II, (f) Laggard at telophase I, (g) Disorientation of chromosome at metaphase II, (h) Polyploidy and (i) Bridges at telophase II

Table 2: Growth and yield studies in $Pb(NO_3)_2$ treated *Lens culinaris* Medik. (M_1 generation)

Concentration (ppm) $Pb(NO_3)_2$	Plant height (cm) Mean±SD CV	Number of branches/plant Mean±SD CV	Number of pods/plant Mean±SD CV	Length/pod (cm) Mean±SD CV	Number of seeds/pod Mean±SD CV	Total no. of seed/plant Mean±SD CV	100-seeds weight (g) Mean±SD CV	Total yield /plant (g) Mean±SD CV
Control	43.26±1.52	3.86±0.49	61.53±1.25	1.06±0.16	2.0±0.36	132.06±2.08	3.10±0.20	4.15±0.45
	3.51	12.69	2.03	15.09	18	1.57	6.45	10.84
5	41.26±3.17	3.66±0.69	61.26±2.04	1.05±0.21	1.80±0.40	120.53**±3.99	3.03±0.31	3.82±0.65
	7.68	18.85	3.33	20	22.22	3.31	10.23	17.01
10	40.00*±3.18	3.53±0.71	60.13±2.47	1.02±0.23	1.73±0.44	112.73**±4.17	2.98±0.34	3.69±0.72
	7.95	20.11	4.1	22.54	25.43	3.69	11.4	19.51
15	38.86**±3.63	3.40±0.80	59.00*±2.63	0.98±0.24	1.66±0.47	104.33**±4.78	2.92±0.37	3.57±0.77
	9.34	23.52	4.45	24.48	28.31	4.58	12.67	21.56
20	35.73**±3.99	3.20*±0.83	57.26**±3.10	0.95±0.25	1.60*±0.48	98.60**±5.63	2.87±0.40	3.31*±0.80
	11.16	25.93	5.41	26.31	30	5.7	13.93	24.16
25	33.33**±4.15	2.93**±0.77	56.40**±3.61	0.92±0.27	1.53*±0.49	92.93**±5.93	2.81±0.42	3.26**±0.85
	12.45	26.27	6.4	29.34	32.02	6.01	14.94	26.07
LSD at 5% (*)	2.98	0.62	2.3	0.18	0.37	4.05	0.3	0.62
LSD at 1% (**)	4.17	0.88	3.22	0.26	0.52	5.67	0.42	0.88

SD: Standard deviation, CV: Coefficient of variations, LSD: Least significant difference

Table 3: Frequency of chromosomal abnormalities induced by $Pb(NO_3)_2$ in *Lens culinaris* Medik (M_1 generation)

Concentration of mutagens (ppm)	Total No. of PMCs	Prophase-I (Diakinesis)			Metaphase-I/II							Anaphase-I/II				Telophase-I/II								
		Univalents	Multivalents	% of abnormalities PMCs	Univalents	Multivalents	Precocious Mov	Stray chromosomes	Stickiness	% of abnormalities PMCs	Laggards	Bridges	Unequal Sep.	% of abnormalities PMCs	Laggards	Bridges	Unequal Sep.	Micronucleate	Multinucleate	Disturbed polarity	Percentage of abnormal PMCs	Total No. of abnormalities PMCs	Total % of abnormalities PMCs observed
Control	289	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$Pb(NO_3)_2$																							
5	262	1	1	0.76	-	1	1	1	2	1.90	2	-	1	1.14	1	-	-	1	-	2	1.52	14	5.32
10	268	2	1	1.11	2	3	3	3	4	5.59	3	1	3	2.61	2	1	1	2	2	2	3.73	35	13.04
15	277	2	2	1.44	3	4	4	4	4	6.85	4	2	3	3.25	3	2	3	3	3	4	6.49	50	18.02
20	249	3	3	2.40	4	5	5	5	5	9.63	4	3	4	4.41	4	3	3	3	4	4	8.43	62	24.88
25	274	4	4	2.91	5	6	6	6	7	10.94	5	4	6	5.47	5	3	5	5	4	5	9.85	80	29.17

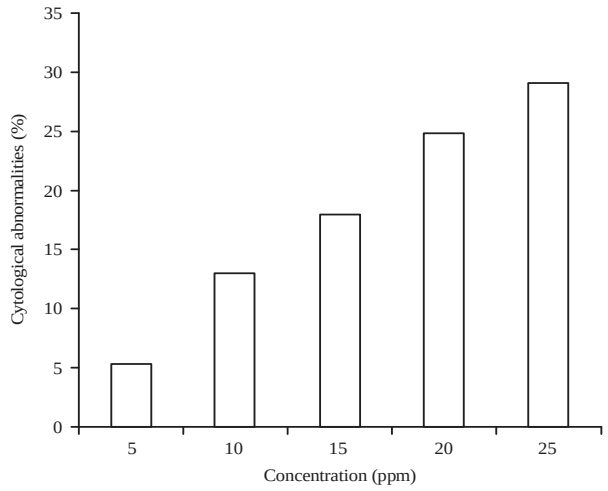

Fig. 2: Percentage of cytological abnormalities induced by $Pb(NO_3)_2$ in M_1 generation

survival was recorded in control (95.0 and 96.84%) and decreased in mutagenized population from 77.00-47.00% and 81.81-70.20% in 5-25 ppm $Pb(NO_3)_2$, respectively (Table 1). Dose dependent decrease in the mean value of all qualitative and quantitative characters such as plant height, branches per plant, pods per plant, seeds per pod, seed weight and total yield per plant etc. was recorded from control in M_1 generation with significant values at 5 and 1% level. The qualitative and quantitative parameters of control and treated population of lentil plant are presented in Table 2. The significant minimally decreased in plant height 38.86±3.63-33.33±4.15 cm, at $p<0.01$ was observed in 15-25 ppm lead nitrate compared to control plant 43.26±1.52 cm (Table 2). A significant minimally decrease in branches per plant was observed 2.93±0.77 at $p<0.01$) in 25 ppm lead nitrate to that of control. A significant decrease was observed in no of pod per plant from lower to higher concentration and total no of seed per plant was significantly decrease in 5-25 ppm $Pb(NO_3)_2$ at ($p<0.01$), respectively over the control 132.06±2.08 (Table 2). Total yield per plant was decreased from lower to higher doses of which 3.31±0.80 minimally significant (at $p<0.05$) in 20 ppm whereas, 3.26±0.85 significant at ($p<0.01$) in 25 ppm of lead nitrate respectively as compared to control.

In the present study, dose dependent increase in meiotic anomalies was observed. Control plant exhibiting normal pollen mother cells in meiotic division in telophase I and anaphase II (Fig. 1a, b) while treated plants showed different type of meiotic lesion such as laggard at anaphase I (Fig. 1c), stickiness at metaphase I (Fig. 1d), disturb polarity with 4 laggard at telophase II (Fig. 1e), laggard at telophase I (Fig. 1f), disorientation of chromosome at metaphase II (Fig. 1g), polyploidy (Fig. 1h) and bridges at telophase II (Fig. 1i).

The frequency of various meiotic abnormalities in treated population with total pollen mother cells percentage in each concentration of lead nitrate has been recorded in Table 3. The maximum frequency of chromosomal aberration was reported in the higher concentration (25 ppm) of lead 29.17, respectively (Fig. 2). The frequency of aberration was dose dependent i.e, its increase with increasing concentration of mutagen. The total percentage of abnormal pollen mother cells varies from 5.32-29.17 in 5-25 ppm of lead nitrate (Table 3) (Fig. 2). Pollen fertility showed very low percentage of sterility pollen in control plants. Pollen fertility was found to be correlated with meiotic aberrations: As the meiotic abnormalities increased along the treatment the pollen fertility decreased (Table 1).

The results of the present investigation show that lead nitrate has a significant inhibitory effect on morphological parameters and chromosomal abnormalities in lentil were dose dependent pattern; these effects were high at higher concentration of lead nitrate (Table 3, Fig. 1).

DISCUSSION

This experiment distinguishes that heavy metal (lead nitrate) showed considerable effect on the phenotypic and genotypic configuration of the plant. In the present investigation, it was observed that seed germination, plant survival were decreased with increasing concentration of heavy metal over the control. Seed germination was inhibited by higher concentration of lead[10-11]. This can be due to the toxic effects of ions on the germination process[12]. According to Ananthaswamy *et al.*[13], reduction in seed germination of treated population is due to inhibition in physiological ad biological processes which is required for seed germination, such as hormonal imbalance and inhibition of meiotic process. Heavy metal (Pb) decrease germination percentage[14], germination index, root and shoot length, tolerance index and dry mass of root and shoot[15-16].

Reduction in survival occured due to various factors such as cytogenetic damage and physiological disturbances[17] and chromosomal aberration which leads to mitotic arrest[18].

Plants of M_1 generation exhibited reduced plant height in the treated population as compared to control in analysis of morphological parameters. This result was also observed by Banu *et al.*[19]. This diminution can be attributed to chromosomal damage. It was recorded by Dimitrova[20], that high concentration of heavy metal inhibited the growth of vegetative organs. According to Dimitrova and Ivanova[21], that high doses of heavy metals present in the soil decreased not only the growth of vegetative organs but also the cell division

rate. In this experiment, the data related yield parameters exhibited a significant reduction in yield parameters. Laxmi and Datta[22] recorded a considerable diminished in yield as compared to control. These results were recorded in soyabean[23], cotton[24], *Trigonella*[25], *Vicia faba*[26] and *Capsicum annum*[27]. It was suggested by Panigrahi *et al.*[28] that increment of yield at significant level and variation in quantitative parameters may demonstrate stable gene mutations in the next generation, while Thilagavathi and Mullainathan[29] assumed that the reduction in quantitative traits have been assigned to the physiological distress or chromosomal destruction due to the cells of plant by mutagen.

Cytological observation describes the specific responses of various kinds of genotypes to a specific mutagen and gives significant confirmation for the selection of desirable traits[30]. In the present inspection, various meiotic lesions like univalents, multivalent, laggard, stickiness, precocious movement, stray chromosome, disturb polarity, disorientation of chromosome, polyploidy, bridges, multinucleate and micronuclei etc. were recorded in mutagenized population of all concentration. According to Srivastava and Srivastava[31], univalents may originate due to an early disjunction of some of partially homologous chromosomes and due to this condition, they are not able to form normal bivalents. Formation of multivalent may also be attributed to the abnormal pairing and non disjunction of bivalents[32]. It was suggested by Jafri *et al.*[33], that precocious movement of chromosome was caused by spindle disfunction. Heavy metal induced comparatively high proportion of stickiness which is caused by partial dissociation of nucleo-proteins and variation in the pattern of cyto-chemically balanced reaction[34]. It was suggested by Minija *et al.*[35], that presence of laggard at anaphase I/II can be attributed due to the delayed terminalization of chiasmata or may be due to stickiness of chromosomal end. Singh and Chaudhary[36] reported that laggard may be found because of breakage or defective spindle which leads to imbalanced daughter nuclei and micronuclei. Disturbed polarity at anaphase and telophase I/II stages could be due to spindle distraction and formed multinucleate condition. Disturbed polarity was also recorded in maize[37], *Trigonella*[38], *Vicia faba*[26]. Bridges formed may be due to non functioning of target proteins which may be caused by gene mutation or direct action of mutagens on proteins creates disruption during separation of chromosome at anaphase and telophase I/II. According to Sinha and Godward[39], formation of brides at telophase may be due to paracentric inversion. This result is also recorded in different plants by various researchers such as in *Cichorium intybus*[40,33], *Trigonella*[25], *Vicia faba*[41], *Capsicum annum*[42,27] and in lentil[43].

Kumar and Gupta[44] treated capsicum seed with heavy metal and observed that a gradual decrease in pollen fertility. Pollen fertility was correlated with meiotic abnormalities. Meiotic abnormalities increased along with the increasing concentration of heavy metal (mercury and cadmium)[45,25]. Similarly decrease in pollen fertility was reported in *Capsicum annum*[46] and *Vicia* faba[26,41].

Mutation breeding study gives an extensive knowledge of the mutagenesis and effect of heavy metal in lentil. This observation suggests that heavy metal used as mutagen to create genetic and phenotypic variation in lentil which could be selected as useful and viable mutant and can be recommended to lentil mutation breeding program.

CONCLUSION

The present research work determines that lead (Pb) had a significant effect on the cyto-morphological characters of *Lens culinaris*. The demonstrated chromosomal lesion represents the effect of pollution on the genetic material and variations in morphology are directly proportional with chromosomal aberrations. It was recorded that low concentration of lead is tolerable by plant without losing viability; while higher concentration is genotoxic and mutagenic to plants.

SIGNIFICANCE STATEMENT

The present study finds out genotypic variation in M_1 generation of lentil by using induced mutagenesis and breeding program. Variation in genotypes may produce different type of variants/mutants plants of lentil with improved qualitative and quantitative characters/traits and selected for further in breeding program for crop improvement. Induced cytological aberration play the huge importance that creates change in the genotypes which can be inherited to the subsequent generation leading into desirable mutations. The recorded results confirm a high phenotypic diversity which has been induced in treated population and the isolated different mutants were of great economic which can be provide as future breeding material in research on lentil.

ACKNOWLEDGMENT

This study was performed at Department of Botany, Aligarh Muslim University, Aligarh. Authors are grateful to chairman for providing the necessary facilities and University

Grant Commission (UGC), New Delhi, India for providing funding under Grant No. F1-17.1/2015-16/MANF-2015-17-UTT-54718/(SA-III/Website).

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