Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Durre Shahwar, Zeba Khan, Mohammad Yunus Khalil Ansari (2022). Cadmium induced genotoxicity and antioxidative defense system in lentil (*Lens culinaris* Medik.) genotype. *Caryologia* 75(3): 47-64. doi: 10.36253/caryologia-1666

Received: May 22, 2022

Accepted: November 23, 2022

Published: April 5, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Cadmium induced genotoxicity and antioxidative defense system in lentil (*Lens culinaris* Medik.) genotype

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Abstract. Induced mutagenesis is considered a coherent mechanism in crop improvement programmes to produce novel plant varieties. Due to the insufficiency of desired genotypes, plant breeders are supposed to re-associate the gene of interest from the accessible gene pool of the related plant species through hybridization to develop new cultivars with desired traits. The present investigation was performed to evaluate cadmium induced mutagenesis on growth performance, physio-biochemical traits and DNA damage studies in lentil. Growth and morphological parameters exhibited reduction with increasing concentration of cadmium. Maximum devaluation was reported at the highest concentration. Physiological and biochemical traits were also affected by different cadmium concentrations and reduced as concentration increased. Lipid peroxidation activity and antioxidant enzymes increased as mutagenic stress increased caused by cadmium. CAT and SOD concentration was found to increase initially and then decreased gradually at higher cadmium concentrations. SEM analysis of stomatal morphology revealed variation in stomatal shape and size in treated populations. There was a gradual enhancement in the percentage of DNA damage along with variation in morphological traits. The DNA damage was recorded as precocious movement, stray bivalent, laggard, stickiness, disorientation of chromosome, multi-bridge, disturbed polarity and micronuclei. It was concluded that at higher concentrations, cadmium cause DNA damage and these chromosomal alterations causes morpho-physiological and biochemical changes in lentil.

Keywords: Abiotic stress, oxidative stress, antioxidant activity, DNA damage, *Lens culinaris*.

ABBREVIATIONS

Cd	Cadmium
CAT	Catalase activity
SOD	Superoxide dismutase

ROS Reactive oxygen species
EDTA Ethyl diamine tetra acetic acid

INTRODUCTION

Nowadays, world is posing a severe threat of malnutrition and food insecurity to human civilization. Scientists are involved in developing new and ingenious approaches to diminish hunger and malnutrition issues which are expanding day by day around the world. Pulses play a significant role in compensating food insecurity, especially for low-income families (Kumar and Pandey 2020).India is one of world's largest producer, importer and consumer of pulses, especially lentils, which have great potential to elucidate the global food crisis. Lentil is highly efficient inadjusting adverse climatic conditions, which resulted in a declaration by United Nations in 2016 as an International Year of Pulses (IYP2016), with interdisciplinary research approaches towards the qualitative and quantitative improvement of pulses.

Lentil is considered as essentially important nutritious crop rich in protein and minerals. *Lens culinaris* is self-pollinated, diploid (2n=14) crop with a genome size of 4063Mbp (Arumuganathan and Earle, 1991). Van Oss *et al.* (1997) suggested that the *Lens* genus has four wild species *L.culinaris*, *L. lamottei, L. nigricans* and *L. ervoides*, whereas (Ferguson et al. 2000) observed that *Lens culinaris* Medikus contain three wild subspecies: *L. culinaris* subsp. Orientalis and *L. culinaris* subsp. *tomentosus* and *L. culinaris* subsp. *Odemensis* of which *L. culinaris* subsp. *orientalis* is considered the ancestor of cultivated lentil. Full knowledge of lentil was given by Barulina (1930), who categorized *Lens culinaris* into two subspecies, of which one is named macrosperma (large seeds with 6-9 mm diameter) and the other microsperma (tiny seeds with 2-6mm diameter). Lentil is known to be a source of protein and high quality fiber among all pulses, because of this property, it is considered an economical food consumed all over the world. Lentil is an accomplished source of essential vitamins and minerals such as foliate vitamin B1, magnesium, phosphorus, potassium, copper complex carbohydrates and vegetable protein and a low amount of fat-free cholesterol (Tharanathan & Mahadevamma, 2003). Lentil contains macronutrients and also poses certain phytochemicals such as; flavonols, phenolic acids, phytic acid, soyasaponins and tannins (Xu & Chang, 2010). It can fix atmospheric nitrogen and increase soil fertility due to increased level of nitrogen in soil and by adding carbon and organic matter. Keeping all these attributes in mind, it becomes necessary to ameliorate lentil variety to obtain genotype of good nutrient quality and yield-related traits. Induced mutagenesis is a helpful technique in the plant-breeding programme for breeders or biological researchers with the embellishment in knowledge of technique for inducing mutation and mutation process itself to produce new cultivar of better quality by creating variability (Chaudhary *et al.* 2019).Mutagenesis has increased genetic variability for qualitative and quantitative traits and induces desirable mutant alleles, which may not previously present in germplasm in a wide variety of species.Induced mutagenesis has played a significant role in overcoming food scarcity for world population and developed new mutant cultivars with increased nutritional values (Suprasanna *et al.* 2015).

Cd is an anthropogenic genotoxic pollutant that is highly soluble in water (Jiang *et al.* 2001)and is readily absorbed by the plants. Cd toxicity reduces uptake and translocation of nutrients and water, increases oxidative damage, disrupts plant metabolism, and inhibits plant morphology and physiology (Haider *et al.* 2021). In plants, primary effect of metal toxicity is inhibition in root growth and cell division, protein denaturation, altered photosynthesis (Rathore *et al.* 2007; Akinci *et al.* 2010) and increases in the frequency of chromosomal aberrations as studied in different plants such as *Allium* by Liu *et al.*, 1994, *Allium sativum* (Yi and Meng, 2003); *Helianthus annuus* (Kumar and Srivastava, 2006); *Lathyrus sativus* (Kumar and Tripathi, 2007a) etc. Heavy metal can induce reactive oxygen species (ROS) (Qian *et al.* 2009). Plants overcome the damage induced via metals stress by activating defense mechanisms which involve both -enzymatic components such as catalase (CAT), superoxide dismutase (SOD) and peroxidase (POX) to protect themselves from ROS (Ruley *et al.*2004) and non-enzymatic components such as glutathione–S-tranferase and glutathione reductase. An increase in ROS causes overproduction of MDA, therefore MDA in plant cell acts as a markerbetween production and scavenging of free radicals. Production of ROS causes oxidative burst in biological macromolecules such as enzymes, proteins, membrane lipids, DNA, chloroplast and carotenoids (Tripathy and Oelmüller 2012). Cadmium binds strongly to DNA and RNA, and alters the DNA transcription process so that DNA synthesis and mitotic activities are disturbed resulting in depolymerization, DNA strand breaks, generation of abnormal nitrogenous bases, DNA – DNA cross-links and DNA – protein cross-links. The present investigation examines cadmium-induced mutagenicity and related stress in lentils by assessing the growth, yield, cytological, physiological and biochemical traits.

2. MATERIALS AND METHODS

2.1 Seed procurement and treatments

Dry, healthy, certified, uniform and equal size seeds of *Lens culinaris* variety L-4076 were obtained from Indian Agricultural Research Institute, New Delhi. Fresh, uniform and healthy seeds of lentil were presoaked in double-distilled water for 24 hours, and the mutagenic treatment of cadmium nitrate were given according to my previous study related to work (Shahwar *et al*. 2019). The comprehensive knowledge of induced mutagenesis and selection of mutant lines are described in detail in earlier study related to the work (Shahwar *et al*., 2022). Presoaked seeds were then subjected to different concentrations (20,40,60,80 and 100ppm) of freshly prepared cadmium nitrate solution in double- distilled water at pH 7.0 for 12 hrs with intermittent shaking after an interval of 1 or 2 hours at room temperature of 25± 2°C. After treatment, the seeds were thoroughly washed with tap water to ensure the removal of adhered metal (Cd^{++}) on the surface of the seed coat.Treated seeds of each concentration were sown in replicates with their respective control in earthen pots having soil mixed with farmyard manure and irrigated regularly.

2.2 Growth and morphological study

The experiment was carried out to demonstrate the cadmium stress on the growth and morphology of *Lens culinaris*. Root and shoot length were measured from randomly selected seedlings of each replicate for 30 days. Agronomical parameters such as plant height, number of branches per plant, yield and yield related traits were recorded during the development.

2.3Determination of physiological and biochemical parameters

2.3.1 Estimation of chlorophyll and carotenoid content

The photosynthetic pigments (chlorophyll a, b and carotenoid) were determined by acetone method (Arnon 1949) following pigment extraction. For the purpose,1 g fresh leaves were ground with 80% acetone and the extract was diluted with double distilled water and the final volume was made 10mL. The optical density (O.D) of photosynthetic pigments were measured at wavelengths of 663 and 645nm (Smith and Benitez, 1955) using UV-VISspectrophotometers. Photosynthetic pigment of the sample was calculated using the following formula:

chlorophyll a = 12.7 (O.D.) 663-2.69(O.D.) 645×v/w×1000
chlorophyll b = 22.9 (O.D.) 645-4.68(O.D.) 663×v/w×1000
Total chlorophyll = 20.2 (O.D.) 645+8.02 (O.D.) 663×v/w×1000
carotenoids = 46.95 (O.D. 440.5-0.268× chlorophyll (a+b)
Where W=fresh weight of extracted tissue in grams
V= total volume of extract

2.3.2 Analysis of stomatal morphology and mineral elements

Stomatal morphology was studied using scanning electron microscopy (JEOL, JSM-6510LV, JAPAN). Scanning electron microscopy andenergy dispersive X-ray microanalysis(EDX) of leaf sample were performed following the protocol proposed by Daud*et al*. (2009) with minor changes. The leaf samples were fixed in 2.5 % glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer (pH 7.0) for 4 hrs and washed for 15 min with phosphate buffer thrice at each step. Leaf samples were then re-fixed for 1 hour with OsO_4 (osmium tetraoxide) in 0.1 M of potassium phosphate buffer (pH 7.0) and were again washed for 15 min with the same phosphate buffer thrice at each step. The dehydration was done after fixation using ethanol series (30%, 50%, 70%, 90%, and 100%) for 15-20 min thrice for each cycle and transferred in the mixture of alcohol and isoamyl acetate (1:1) for half an hour and in pure isoamyl acetate for one hour. Dehydration of specimens were done by Zeiss Evo 60 (Carl Zeiss SEM, Germany) critical point dryer using liquid carbon dioxide, the samples were coated with a thin layer of Palladium and observed under SEM at 15 kv with x1500 magnifications. Prepared leaf samples were analyzed through EDX for mineral element analysis.

2.3.3 Estimation of proline content

Leaf sample was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and centrifuged at 9000 rpm for 10 min. 2ml glacial acetic acid was added to 2 mL of supernatant; further 2ml ninhydrin solution in 30ml acetic acid and 20mL of 6M H_3PO_4were added. The solution was incubated at 100°C for 1 hour and OD was recorded at 520 nm using toluene as blank. Proline content in test sample was calculated using a standard curve (Bates *et al*. 1973).

2.3.4 Determination of lipid peroxidation/MDA content

Malondialdehyde (MDA) content was measured following the protocol proposed by Hodges *et al*. (1999) and expressed as μ moles $g^{-1.}$

2.3.5 Antioxidant enzyme activity assay

Antioxidant enzyme assay was done by the method proposed by Sinha *et al.* (2018) with slight modification. Fresh leaves tissues were grinded in 1 ml extraction buffer having 80 mM sodium phosphate buffer, 1mM EDTA, 1 m Mphenylsulfonylfluride (PMSF), 1% polyvinyl pyrrolidone (PVP), and 0.5% (v/v) Triton X-100 and centrifuged at 11000 rpm for 25 min at 4°C. The supernatant kept at -20°C was used to determine antioxidant enzyme activities such as catalase (CAT) following protocol proposed by Yu and Rengel (1999), superoxide dismutase (SOD), Gallego *et al*. (1996) and peroxidase (POX) Kar and Mishra (1976).

2.3.6 Estimation of protein content

Dry seeds (0.5g) were ground in 10ml water and 1ml of 10%trichloroaceticacid was added to the extract. The sample was kept in an ice bath for 10 min. and the supernatant was collected and centrifuged at 5000 rpm for 10 min at 4°C. 20 ml sodium hydroxide (0.1N) was added to dissolve the protein and the total volume was made the nearest whole number. Seed protein content of the extract was determined by Lowry's method (1951) using BSA (Bovine serum albumin) as standard and absorbance were measured at 650 nm.

2.4 DNA damage Studies

For chromosomal studies, young and small-sized flower buds were collected from treated and control plants, fixed in freshly prepared Carnoy's fluid (1:3:6 ratio of glacial acetic acid, chloroform and alcohol) and were preserved in 70% alcohol. For DNA damage studies,anthers of appropriate size were squashed in 0.5% propionocarmine stain, dehydrated in normal butyl alcohol series and mounted on Canada balsam to prepare permanent slides. Microphotographs of chromosomal lesion or DNA damage were taken from temporary and permanent slides by "Olympus" microphotographic unit.

2.5 Statistical interpretation

The results were analyzed and interpreted statistically using software SPSS version 20 for windows 10 using one-way ANOVA. For determinationof least significant difference (LSD) at 5% and 10% probability ($p < 0.05, 0.01$), data analysis of variance, one-way ANOVA was done using Duncan's Multiple Range Test (DMRT) (Duncan, 1955)

3. RESULTS

3.1 Effect of heavy metal stress on growth and morphological parameter

3.1.1 Germination, survival and pollen fertility

Effects of cadmium stress on seedling growth were investigated on 15 days old seedling. It was observed that plant germination, survival and pollen fertility decreased linearly in dose-dependent manner. The inhibitory effect on germination and related parameters were evident at the highest concentration of heavy metal. Fig. 1A depicts a gradual decrease in these characters as concentration increases. The highest concentration (100 ppm) of mutagen exhibited a maximum reduction in all these parameters.

3.1.2 Effect of Cd heavy metal on root and shoot length (cm)

A more pronounced impact of cadmium stress on root and shoot lengths were observed in treated plants. Fresh weight of the seedlings decreased significantly with increase in cadmium concentration. The decrease was significantat 80 and 100 ppm for root length and in 40-100 $Cd(NO_3)_2$ for shoot length.Inhibitory effect on the seedling growth was higher in the root than in the aerial segment. (Fig. 1B).

3.1.3 Plant height

At maturity plant height was found to be maximum in control 43.26±1.52 and decreased significantly from 39.53±3.24 to 31.80±3.31 in 20 to100 ppm both at 5% ($p < 0.05$) and 1 % level ($p < 0.01$) (Table 1).

3.1.4 Number of branches per plant

Mean for number of branches per plant was found to be 3.86±0.49 in control and decreased significantly at

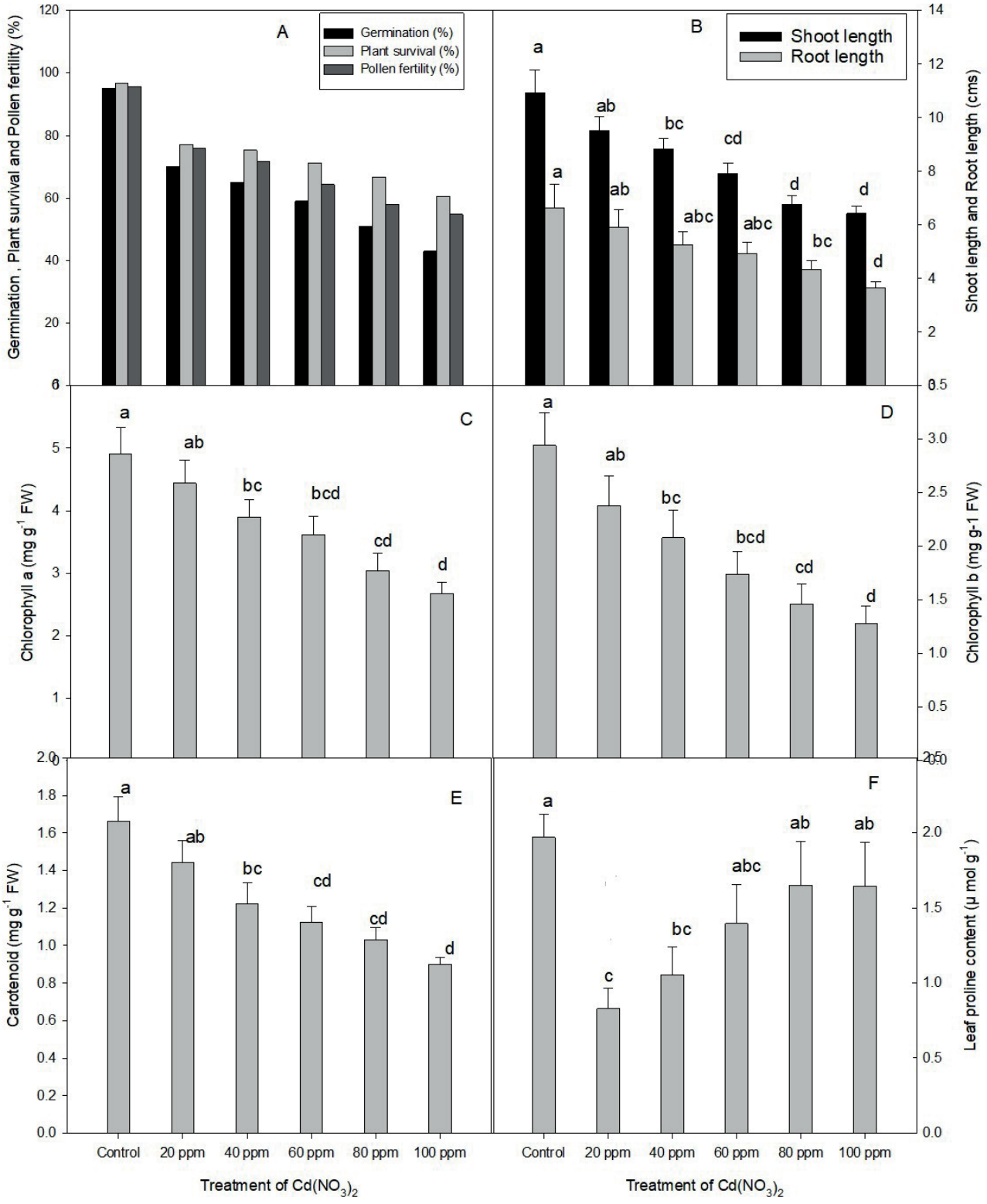


Figure 1. Effect of $Cd(NO_3)_2$ on germination, survival, and pollen fertility, root and shoot length (cm), photosynthetic pigments and proline content (µmoles/g dry wt) in *Lens culinaris*. Medik L. (M_1 generation). Data means within columns followed by the same letter is not different at the 5% level of significance, based on the Duncan Multiple Range Test.

Table 1. Growth and Yield Studies in $Cd(NO_3)_2$treated *Lens culinaris* Medik.

Conc. ppm $Cd(NO_3)_2$	Plant Height (cm) Mean±SD CV	No. of Branches/Plant Mean±SD CV	No. of Pods/Plant Mean±SD CV	Length/pod (cm) Mean±SD CV	No. of Seeds/ pod Mean±SD CV	Total no. of Seeds/Plant Mean±SD CV	100-Seeds Weight (g) Mean±SD CV	Total Yield/ plant(g) Mean±SD CV
Control	43.26±1.52 3.51	3.86±0.49 12.69	38.53±1.25 3.24	1.06±0.16 15.09	2.0±0.36 18.0	77.06±2.08 2.69	3.10±0.20 6.45	2.38±0.45 18.90
20	39.53*±3.24 8.25	2.93**±0.57 19.45	37.26±2.48 6.65	1.00±0.23 23.00	1.66±0.44 26.50	61.85**±4.42 7.14	2.94±0.36 12.24	1.81±0.69 38.12
40	38.93*±3.31 8.50	2.73**±0.67 24.54	36.13±2.67 7.38	0.96±0.24 25.00	1.46*±0.48 32.87	52.74**±4.64 8.79	2.88±0.39 13.54	1.51*±0.78 51.65
60	35.00**±4.22 12.05	2.66**±0.73 27.44	34.46**±3.36 9.75	0.92±0.25 27.17	1.33**±0.49 36.84	45.83**±5.15 11.23	2.80±0.41 14.64	1.28**±0.81 63.28
80	32.46**±4.68 14.41	2.40**±0.80 33.33	32.53**±3.79 11.65	0.87 ±0.27 31.03	1.26**±0.49 38.88	40.98**±5.81 14.17	2.72*±0.46 16.91	1.11**±0.75 67.56
100	31.80**±4.96 15.59	2.26**±0.78 34.51	31.66**±4.09 12.91	0.84*±0.28 33.33	1.20**±0.48 40.00	37.99**±6.09 16.03	2.68*±0.50 18.65	1.01**±0.70 69.30
LSD at 5% (*)	3.37	0.60	2.70	0.20	0.41	4.28	0.34	0.64
LSD at 1% (**)	4.72	0.84	3.78	0.29	0.59	5.99	0.48	0.90

SD= Standard Deviation, CV= Coefficient of Variations, LSD= Least Significant Difference.

1% ($p < 0.01$) from lower to higher concentration. Coefficient of variation increased with the increasing concentration of mutagens (Table 1).

3.1.5 Yield attributing traits

Number of pods per plant, number of seeds per pod, total number of seeds per plant, 100 seed weight and total yield per plant are the yield related traits. All these parameters were found to reduce significantly at 5% ($p < 0.05$) and 1% level ($p < 0.01$) when compared with their respective control (Table-1). Number of pods per plant decreased significantly at 1% level ($p < 0.01$) from 34.46±3.36 to 31.66±4.09 (60-100 ppm) concentration and the number of seeds per pod decreased at 1% level in 60-100 ppm $Cd(NO_3)_2$ (Table-1). Total number of seeds per plant, 100 seed weight and total yield per plant significantly decreased minimally from lower to higher doses of cadmium nitrate. Coefficient of variation increased with increasing concentration of cadmium which means the coefficient of variation is directly proportional to the concentration of mutagen.

3.2 Physiological and biochemical study

3.2.1. Photosynthetic pigment

Estimation of photosynthetic pigments revealed some significant variations in control and treated plants (Fig. 1C-E). Photosynthetic pigments reduced as Cd concentration increased. Chlorophyll 'a', 'b' and carotenoid significantly decreased from 40-100 ppm and the maximum reduction was recorded at highest concentrations-with minimum chlorophyll contents.

3.2.2 Proline content

Proline content increased remarkably by Cd exposure. Lowest concentration of proline was observed at 20 and 40 ppm, i.e. 2.12 and 2.35 μ moles/g fw, respectively compared to the other treatments, (Fig. 1F) while its production enhanced insignificantly with the increasing concentrations. Maximum significant increase in proline concentration (3.24 μ moles/g fw) was recorded at 100 ppm. Increased proline concentrations are common symptoms of metal stress and served as a non-specific index of Cd-toxicity.

3.2.3 Lipid peroxidation assay

Estimation of lipid peroxidation was done by determining the malondialdehyde content in control and cadmium stressed plants. The MDA content enhanced significantly in all concentrations over the control. The maximum increase of MDA content was 1.10 μ M g^{-1} at 100 ppm of $Cd(NO_3)_2$ (Fig. 2A).

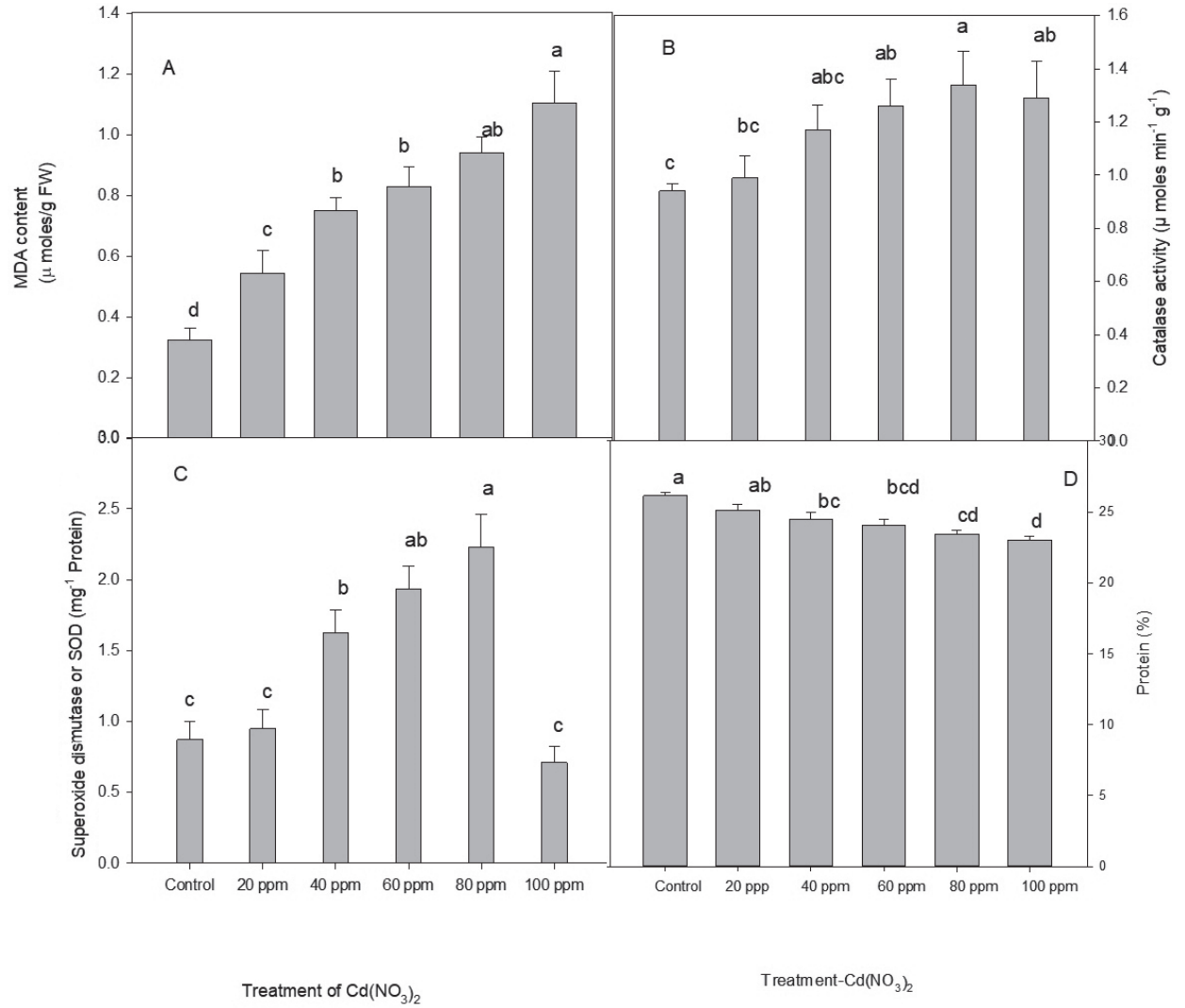


Figure 2. Effect of different concentrations of $Cd(NO_3)_2$ on lipid peroxidation (MDA content μmoles/g FW), catalase activity (CAT) (μmoles $min^{-1}g^{-1}$) and superoxide dismutase (SOD) (U mg^{-1} Protein) and protein content (%) in *Lens culinaris* Medik. Data means within columns followed by the same letter is not different at the 5% level of significance, based on the Duncan Multiple Range Test.

3.2.4 Antioxidant enzyme activities

Antioxidant activity (CAT, SOD) in leaves were found disturbed under cadmium stress. The antioxidant enzyme activity of lentil was found to be increased initially and then fall at higher doses. The catalase activity increases insignificantly over control in 20 and 40 ppm cadmium whereas it increased significantly in 60-100 ppm (Fig. 2B). On the other hand, SOD activity was significantly enhanced at 40-80 ppm cadmium respectively and thereby decreases (0.71 mg^{-1} protein) with their respective control (0.87 mg^{-1} protein) at 100 ppm (Fig. 2C).

3.2.5 Estimation of protein content

Result of estimation of protein content in *Lens culinaris* is depicted in (Fig. 2D). Protein content decreased as cadmium concentration increased. Highest concentration (100 ppm) showed lower percentage of protein (23.0%) over control. An inverse relationship between cadmium concentration and protein content was observed. Statistical analysis shows a significant difference in each treatment except 20 ppm of Cd at ($p < 0.05$).

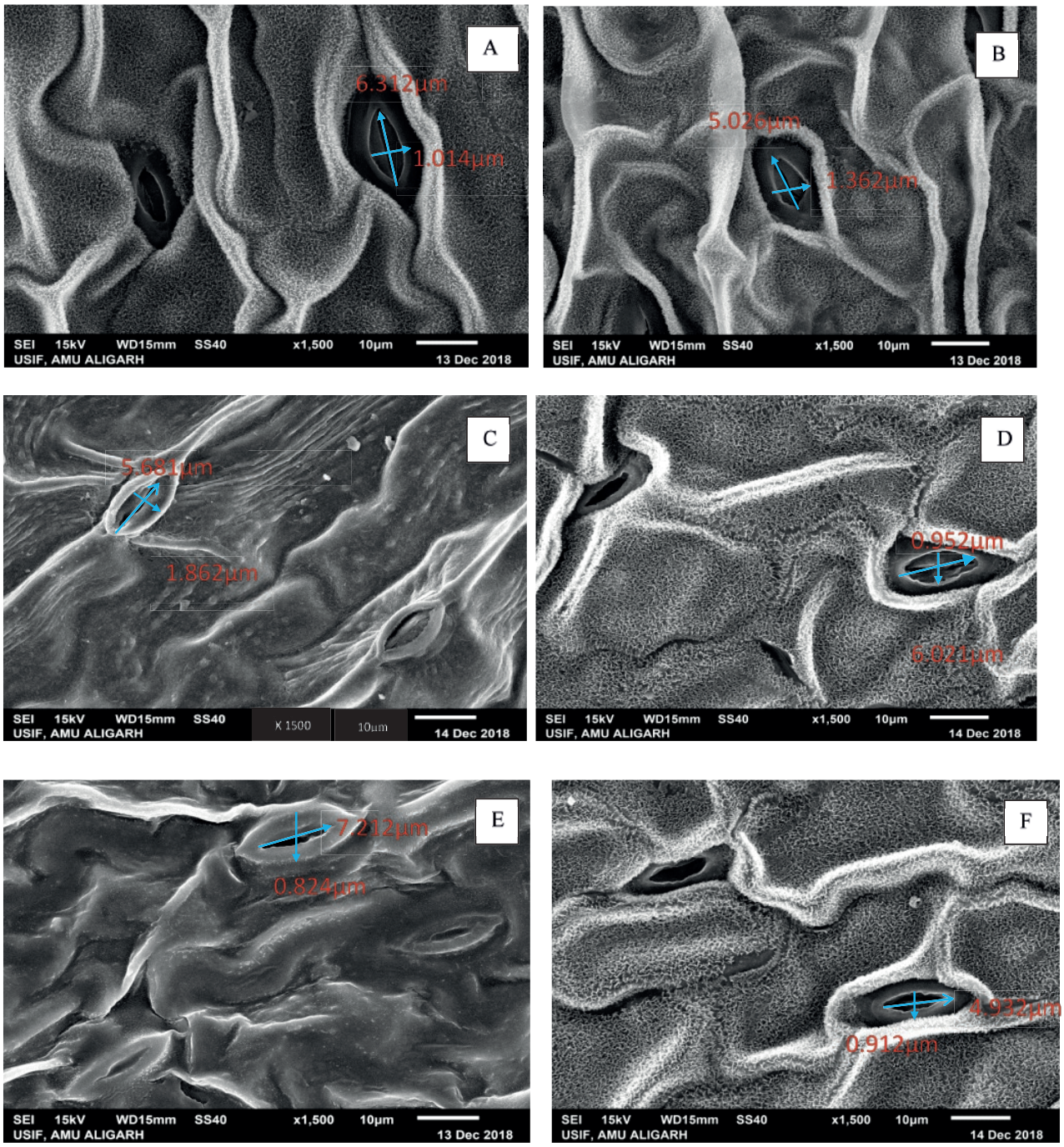
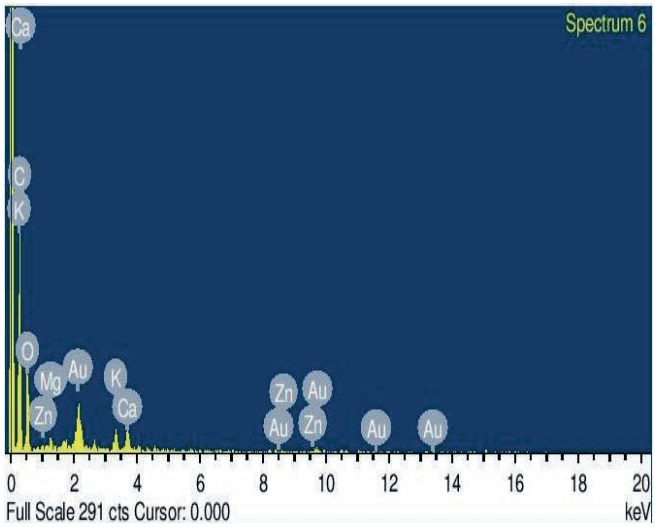


Figure 3. Scanning electron micrographs exhibiting morphology of stomata in control (A) and different shape and size of stomata in various concentrations of cadmium nitrate (20-100 ppm) (B-F).

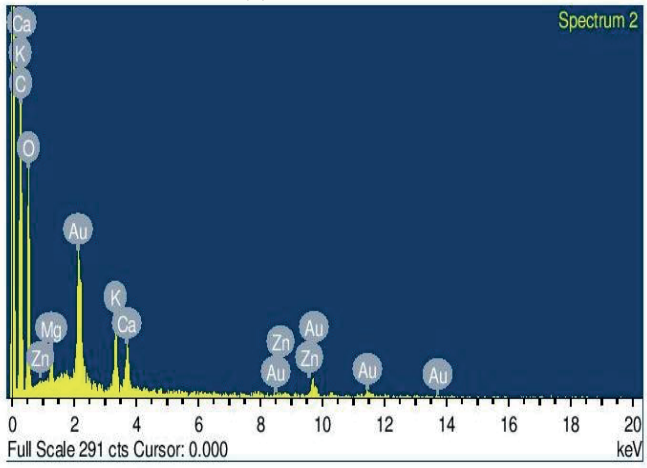
3.2.6 Stomatal behavior and mineral element analysis

Variation in structure of guard cells in treated populations was determined through scanning electron microscopy (SEM). The SEM image showed variation in shape, length and width of guard cells in treated populations. Cadmium treatment induced partially closed stomata. Stomatal opening slightly increases over control in lower doses while it reduced in higher doses with their respective control (Fig. 3; a-f). EDX profiling of leaf was



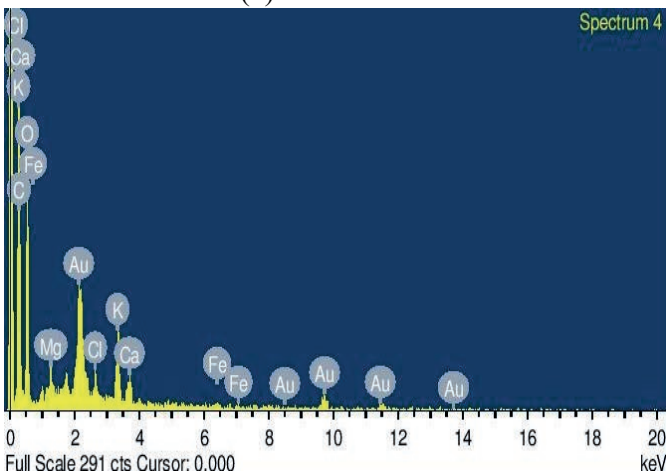
(a)

Element	Weight %	Atomic %
C K	47.80	60.92
O K	37.58	35.96
Mg K	0.89	0.56
K K	1.98	0.78
Ca K	2.44	0.93
Zn K	0.77	0.18
Au M	8.53	0.66



(b)

Element	Weight %	Atomic %
C K	44.04	58.09
O K	38.97	38.58
Mg K	0.95	0.62
K K	2.40	0.97
Ca K	1.99	0.79
Zn K	0.12	0.03
Au M	11.54	0.93



(c)

Element	Weight %	Atomic %
C K	33.56	46.67
O K	46.44	48.48
Mg K	0.93	0.64
Cl K	1.20	0.57
K K	4.35	1.86
Ca K	1.58	0.66
Fe K	0.53	0.16
Au M	11.41	0.97

Figure 4. EDX profiling of mineral content of leaf (a) control; (b) 40 ppm Cd; (c) 80 ppm Cd.

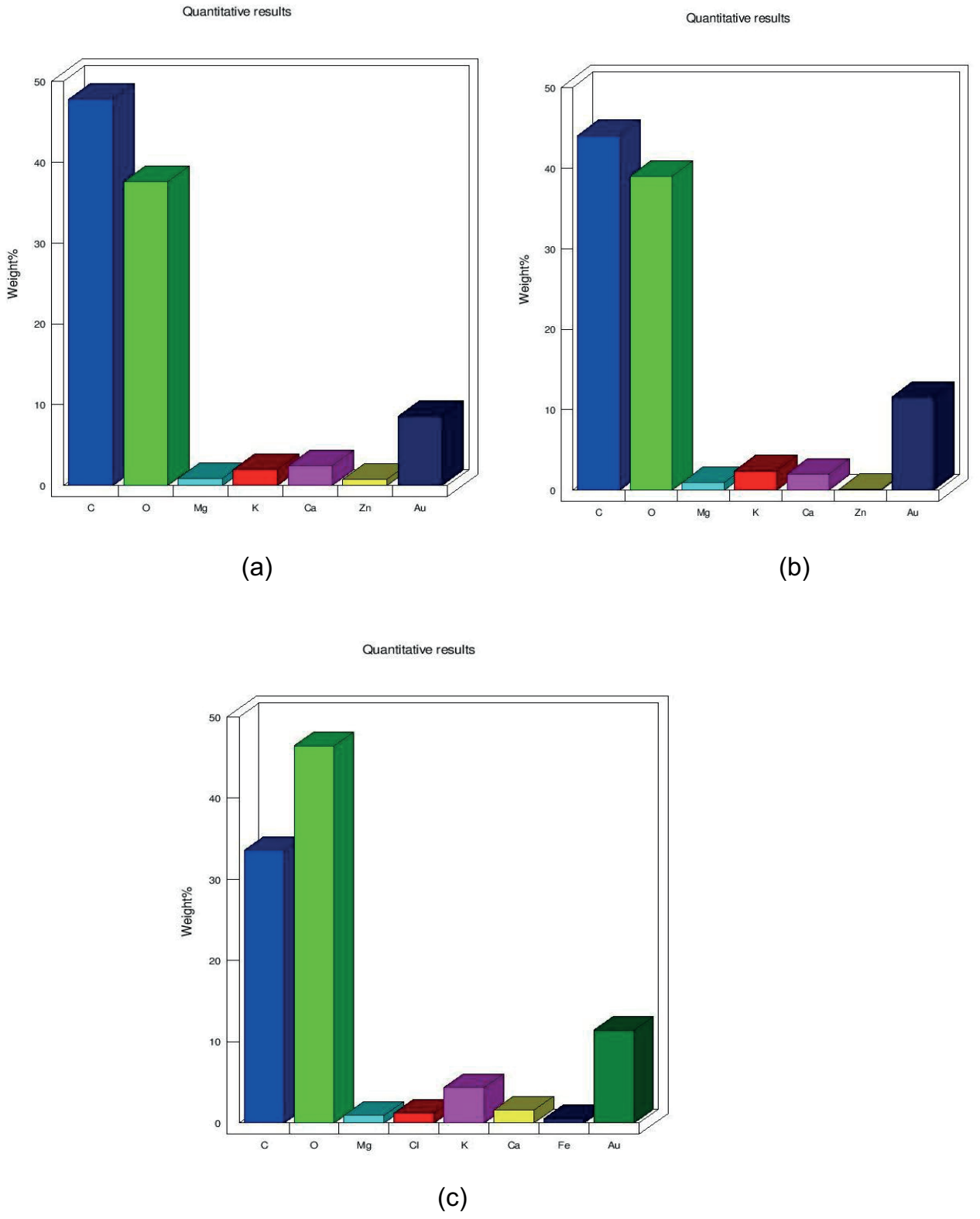


Figure 5. Graphical representation of EDX profiling of mineral content of treated plant of lentil along with control plant.

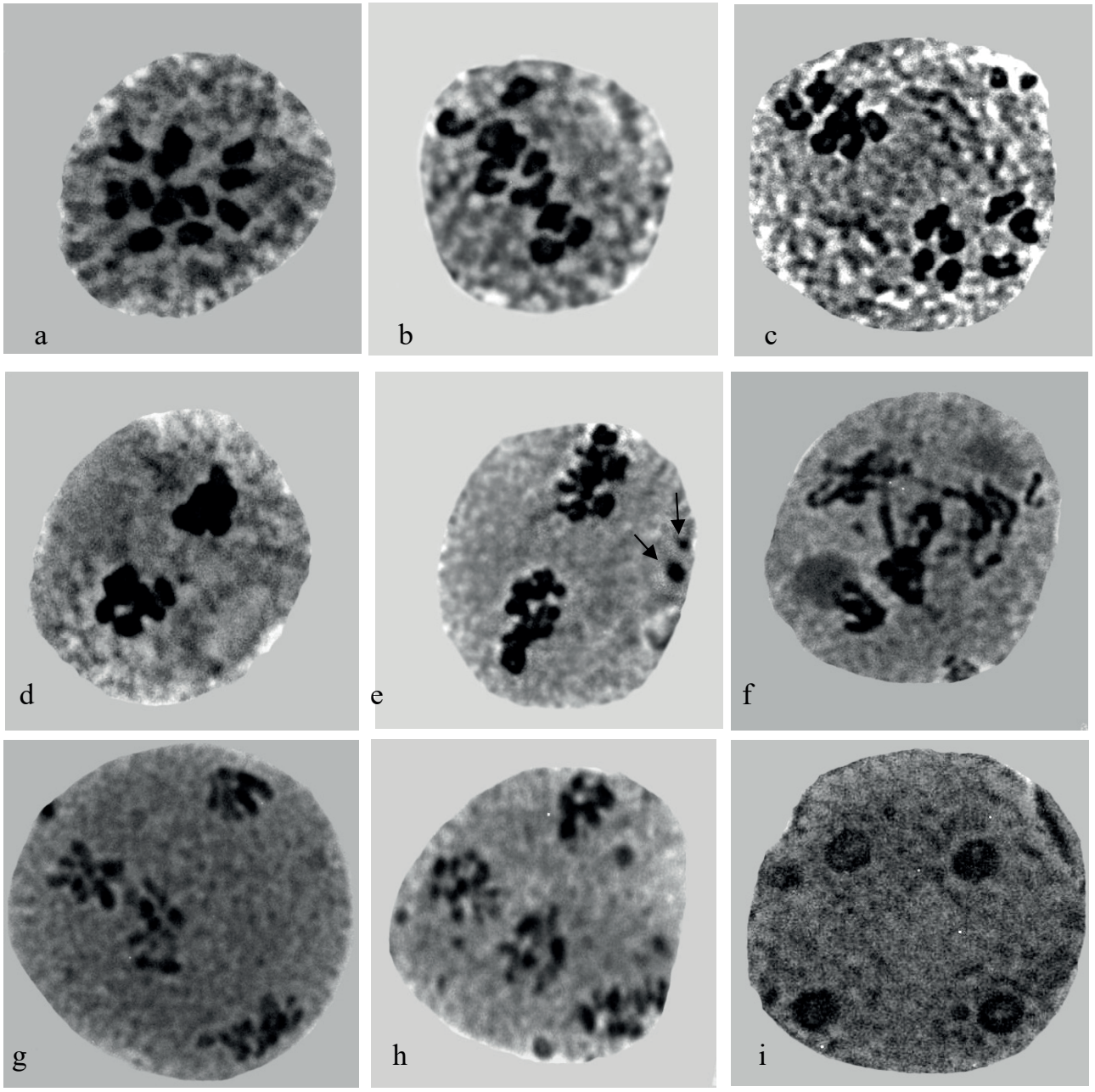


Figure 6. a: Metaphase I (control), b: Metaphase I (precocious movement of chromosome), c: Anaphase I (unequal division with two laggards), d: Telophase I (sticky chromosomes), e: Metaphase II (stray chromosomes), f: Anaphase II (disturbed polarity with multi bridge formation) g, h: Anaphase II (disturbed polarity), i: Telophase II (two micronuclei).

also done via energy dispersive X-ray analyser (EDX)to estimate mineral element of control as well as treated plants. Treated populations exhibited a slight reduction and enhancement in mineral elements as compared to control when expressed in percentage content (Fig. 4 and 5a-c)

3.3 DNA damage

Meiotic studies in pollen mother cells treated with different concentrations of Cd are shown in Fig. 6. The aberrant cells increased as heavy metal concentrations increased. Untreated plants exhibited normal meiotic cells at metaphase I (control) (Fig. 6a).Various chromo-

Table 2. Frequency of chromosomal anomalies induced by $Cd(NO_3)_2$ in *Lens culinaris* Medik. (M_1Generation).

	Prophase-I (Diakinesis)			Metaphase-I/II					Anaphase-I/II					Telophase-I/II							
Conc .of mutagen (ppm)	Total no. of PMCs observed	Univalents	Multivalents	% of Abn. PMCs (A)	Univalents	Multivalents	Precocious Mov. of chromosomes	Stray chromosomes	Stickiness	% of Abn. PMCs (B)	Laggards	Disturbed polarity	Unequal Sep. of chromosomes	% of Abn. PMCs (C)	Laggards	Bridges	Unequal Sep. of chromosomes	Micro nucleate cells	Multi nucleate cells	Disturbed polarity	Cytomixis	% of Abn. PMCs (D)	Total No. of Abnoral PMCs observed	Total % of Abnormal PMCs observed A+B+C+D
Control	289	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
$Cd(NO_3)_2$ (ppm)																								
20	270	2	2	1.4	1	1	2	1	2	2.59	2	1	2	1.85	2	-	-	2	1	2	1	2.96	24	8.88
40	265	3	2	1.8	3	2	4	3	3	5.66	3	2	4	3.39	3	1	1	3	2	3	2	5.66	44	16.59
60	261	2	3	1.9	3	3	5	4	5	7.66	5	3	4	4.59	4	2	2	3	3	4	3	8.04	58	22.20
80	250	3	4	2.8	4	4	6	5	7	10.4	6	4	6	6.40	5	3	3	4	4	5	5	11.60	78	31.20
100	245	4	5	3.6	5	6	8	6	8	13.46	7	4	7	7.34	6	3	4	5	5	6	5	13.87	94	38.34

Figure 7. Effect of $Cd(NO_3)_2$ on percentage of total chromosomal aberrations in *Lens culinaris* Medik.

somal anomalies in pollen mother cells of treated populations were observed, such as precocious movement of two univalents at metaphase I (Fig. 6b), unequal division with two laggards at anaphase I (Fig. 6c), stickiness at telophase I (Fig. 6d), stray chromosomes at metaphase II (Fig. 6e), disturb polarity with multi bridge formation at anaphase II (Fig. 6f), disturbed polarity at anaphase II (Fig. 6g), laggards at telophase II (Fig. 6h), two micronuclei at telophase II (Fig. 6i). In the present investigation, chromosomal aberrations and frequency of meiotic abnormalities at each concentration were calculated in percentage (Table 2). Maximum frequencies of chromosomal aberrations were observed at 100 ppm. The total percentage of abnormal PMCs ranged from 8.88 to 38.34% (Table 2, Fig. 7)

4. DISCUSSION

As reported earlier by many researchers, Cd is a non-essential element that is readily taken by plants and inhibits plant physiological processes such as water absorption, photosynthesis, stunted foliage, withering of leaf and alters normal meiotic division (Patra *et al.* 2004).The present study showed that exposure of lentil genotypes to different doses of heavy metal (Cd) exhibited substantial alterations in the phenotypic and genotypic makeup of the plant. During growth and develop-

mental stages, morpho-physiological parameters were examined as well as biochemical parameters, antioxidant enzymes activity, DNA damage, SEM and EDX analysis of leaf were also performed to evaluate the overall effect of Cd on plant ecology.

4.1. Growth and morphology

4.1.1 Seed Germination, Survival and pollen fertility

Germination percentage, survival and pollen fertility were found to decrease as cadmium doses increased in the present investigation. Similar observations were reported by (Choudhary*et al.* 2012) in *Trigonella*, (Shahwar *et al.* 2016) in *Vicia faba* and (Shahwar *et al.* 2018, Sharma *et al.* 2022) lentil, (Petrescu *et al.* 2020) in *Ocimum*. Inhibition in germination and root development was due to Cd (Pandit and Prasannakumar 1999) low water uptake, reduction in cell division and metabolic activity and enlargement of the embryo. It was reported by (Moreno *et al.* 1999) Cd disrupts the uptake of water and nutrients in plants and suppresses cell division (Liu *et al.* 2003). Kabir *et al.* (2008) and Farooqi *et al.* (2009) suggested that inhibition in germination percentage, seedling length, tolerance index and dry mass of root and shoot is due to heavy metal. The reason behind reduction in germination percentage under Cd stress might be due to escalated breakdown of reserved food material in seed embryo. Depletion in survival may be due to different cytological and physiological disturbances (Girija *et al.* 2013) and inability to maintain balance between growth regulators and promoters (Meherchandani 1975). The descending fertility is an outcome of chromosomal breakages and anomalies which affect microsporogenesis leading to generation of non-viable gametes and decreasing plant fertility (Kumar and Singh, 2020).

4.1.2 Root and shoot length

In the present investigation, root and shoot lengths minimized linearly as Cd doses increased. Similar result was also reported by Choudhary *et al.* (2012). Decrease in seedling length following metal treatment might be due to reduction in meristematic cells and also due to alteration in hydrolytic enzymes; sufficient food does not reach the developing radical and plumule, resulting in stunting of seedlings (Shafiq *et al.* 2008). According to Elloumi *et al.* (2007), effect of Cd exposure on root growth was more compared to shoot growth since roots are the first organ to contact the heavy metal and carry out the process of absorption (Guilherme *et al.* 2015)

4.1.3 Plant height and yield attributing traits

In the present work, metal treated plants exhibited linearly declined plant height in comparison to the control plants and this depletion was due to chromosomal damage. Reason behind the yield depletion was meioticturbulences whichaffected the production of normal microspores and megaspores resulting in low fruit set. Higher concentration of Cd causes growth inhibition which ascribes to cell division or various desecrations in the plant genome. Thilagavathi and Mullainathan (2011) reported that adecrease in quantitative traits have been ascribed to the physiological perturbation or due to chromosomal breakage. Yield is considered an important agronomical parameter in breeding program.Data regarding yield and related traits, exhibited significant decrease in yield at higher concentrationwhich might be due to metal induced genotoxicity resulting in alterations of physiological mechanisms, chromosomal aberrations and high pollen sterility.

Similar results were recorded in soyabean (Pavadi and Dhanavel, 2004), cotton (Sundaravadivelu *et al.* 2006) *Trigonella* (Choudhary *et al.* 2012), *Vicia faba* (Shahwar *et al* 2016) and *Capsicum annum* (Aslam *et al.* 2017).

4.2. Physio and biochemical aspects

4.2.1 Photosynthetic pigment

Photosynthetic pigment is an important parameter directly correlated with plant growth and biomass (Acosta-Motos *et al.* 2017). In our study, photosynthetic pigment was inversely proportional to cadmium doses, their content decreased with enhancing concentration of cadmium relative to the control. Zengin and Munzuroglu (2006) and Elloumi *et al.* (2007) demonstrated the same result in sunflower and almonds, respectively. The decline in the chlorophyll content in plants might be due to suppression of enzymes such as δ-aminolevulinic acid dehydratase and protochlorophyllide reductase (Van Assche and Clijsters 1990), which are necessary for chlorophyll biosynthesis. Lee*et al.* (2004) and Siler *et al.* (2007) while working on *Paspalum vaginatum* (L.) and *Centaurium erythraea* (L.) respectively reported that total chlorophyll diminished along with the enhanced metal concentration. Carotenoids are an important constituent of photosynthetic pigments which absorb light energy to make food for plant. Carotenoids also save chlorophyll from photo damage. In the present study, photosynthetic pigment, stomatal length and width reduce by cadmium treatment. This reduction is probably due to nutritional imbalance (Wong and Wong 1990).

4.2.2 Proline content

Proline, a non-enzymatic antioxidant, scavenger of ROS, which accumulates in plants when exposed to abiotic stress (Saradhi *et al.* 1993). Itis considered as stress signaling molecule having capability to act as an antioxidative defense molecule. (Maggaio *et al.* 2002). It was reported by researchers that proline accumulation might act as compatible osmolyte in cells, maintains the configuration of macromolecule and organelles and its enhanced production confirms the osmo-tolerance in plants (Nanjo *et al.* 1999; Junaid *et al.* 2008). Dhir *et al.* (2004) demonstrated that proline accumulates in shoots of higher plants such as *B. juncea, T. aestivum and Vigna radiata* in response to Cd toxicity.

4.2.3 Protein content

In present investigation, it was observed that cadmium treatments affected greatly protein synthesis. A significantnegative difference was seen between treated plants and control. Similar results were also found by Bavi *et al.* (2011) in pea plants and Choudhary *et al.* (2012) in *Trigonella*. Balestrasse *et al.* (2003) reported that decline in protein content might be due to inhibition in protein synthesis or an increase in the rate of protein degradation. Higher concentration of cadmium inhibits protease activity and total protein content. This shows toxic effect of cadmium concentration on mechanism of protein synthesis resulting in decreased protein content. Despite of these Chen *et al.* (2007) found that protein content decreased in *Vigna unguiculata* under the salt stress (sodium chloride).

4.2.4 Antioxidant and lipid peroxidation

Heavy metal stress may have detrimental effects on plant stress machinery. Andre *et al.* (2010) suggested that antioxidant enzymes are considered an essential defense element against stress and improve the activity of antioxidant system to overcome stress generated by ROS. ROS are known as the natural by-products of aerobic organisms and are generated during mitochondrial electron transport (Debnath *et al.* 2021). In the present investigation, dose-dependent enhancements in antioxidant enzyme activity were recorded, suggesting ROS production due to severity of Cd stress. Salama *et al.* (2009) and Shehab *et al.* (2010) observed that antioxidant activity elevates as concentration increases but decreases at higher concentrations, probably due to chronic stress exposure. SOD plays a crucial role to safeguard plants against stressby converting O_2^- to H_2O_2 with the help of POX and subsequently reducing it into H_2O (Alscher *et al.* 2002). The results are supported by Arleta *et al.* (2001); Dixit *et al.* (2001); Choudhary *et al.* (2012). Elevated malondialdehyde (MDA) levels indicated enhanced lipid peroxidationincreasing concentration of Cd confirming metal induced oxidative stress in lentil plant. Similar results are recorded by Malecka *et al.* (2001); Unavyar *et al.* (2006).

4.3. DNA damage

Chromosomal anomalies are induced due to factors that affect DNA synthesis and replication or on nucleoproteins, resulting in chromosomal breakages or malfunctioning of spindle apparatus and abnormal chromosomal segregation (Sutan *et al.* 2018). In our investigation, adverse effect of cadmium on the frequency of chromosomal anomalies were observed, presumably due to mutagenic effect of subject heavy metal in inducing alterations in DNA. While we observed normal meiotic cells in control group, a spectrum of anomalies was observed in treated individuals. The frequency of chromosomal aberrations was directly proportional to the concentration of cadmium. The anomalies induced by cadmium nitrate were of broad spectrum and comparatively included a higher proportion of sticky chromosomes. Khan *et al.* (2012) suggested the occurrence of sticky chromosome as a result of improper folding of chromosome fibers and their intermingling. Jayabalan and Rao (1987) reported that stickiness was caused by the segregation of histone proteins and alterations in the pattern of cyto-chemically balanced reactions. Bhat *et al.* (2007) suggested that stray chromosomes may be due to spindle dysfunction and clustering of chromosomes. Anaphasic bridges originate due to unequal separation of dicentric chromosomes (Singh and Khanna, 1988) or presence of sticky chromosomes which remain connected by chromosome bridges during anaphase because of incomplete separation of the daughter chromosomes (Kabarity *et al.* 1974). Laggards were observed at anaphase and telophase in Cd treated plants, and it originates due to disruption of spindle. Das and Roy (1989) hold the view that spindle fibers fail to carry chromosomes to their respective poles due to mutagen reaction leaving the chromosome behind as a lagging chromosome or laggard. Stickiness of chromosomal end, delayed terminalization and failure of chromosomes to move at opposite poles were also possible reasons behind laggard production (Verma *et al.* 2012). Disturbed polarity at anaphase and telophase might be attributed to disturbances in the spindle fibers (Bhat *et al.* 2007).

Utsunomiya *et al.* (2002) had opinion that formation of micronuclei is because of non-oriented chromosomes which are unable to reach the pole. Ruan *et al.* (1992) suggested that micronuclei are kind of abnormality which culminates into loss of chromosomal material and is regarded as an indicator of mutagenicity.

Our result suggested a close colinearity between the treatments and percentage of chromosomal anomalies, higher the concentration, more the damage chromosome undergoes. Similar observations were also reported by treatment of different metals and chemicals by other workers such as Srivastava and Kapoor (2008); Khan *et al.* (2009b); Kumar and Yadav (2010);Tripathi and Kumar (2010); Jafri *et al.*(2011); Gulfishan *et al.* (2012); Shahwar *et al.* (2016, 2017, 2018, 2019, 2020); Aslam *et al.* (2017), Khan *et al.* (2019).

5. CONCLUSION

During the present investigation, it was concluded that cadmium induced morphological, physiological, biochemical variation and DNA damage over control in *Lens culinaris.* Genotypes of lentils were greatly affected due to the treatment of cadmium, recommending genetic variation in the subsequent generation. It was observed in this study that at their lower concentrations, cadmium was tolerable by the plant without losing viability, while higher concentrations were genotoxic and induce variation/mutation in the genotypes as well as phenotypes and causing more variation and developing variants/mutant of better quality and selected it. Therefore, plants with better characteristics should be isolated and selected for crop improvement programmes. Further molecular techniques or various genetic engineering techniques should be carried out to check the mutation at genic level as it will be acoherent tool to isolate the desired characters and produce a new variety of lentil through breeding program.

ACKNOWLEDGEMENT

Authors acknowledge their sincere thanks to the Chairman, Department of Botany, Aligarh Muslim University, Aligarh for furnishing all necessary facilities required to perform the above research, and very grateful to the IARI, New Delhi for providing lentil seeds to perform this study. D.S. is thankful to University Grant Commission (UGC), New Delhi for granting research fellowship under MANF scheme.

REFERENCES

Acosta-Motos JR, Ortuño MF, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco MJ, Hernandez JA (2017) Plant responses to salt stress: Adaptive mechanisms. Agronomy 7:18.

Akinci IE, Akinci S, Yilmaz K (2010) Response of tomato (*Solanum lycopersicum* L.) to lead toxicity: Growth, element uptake, chlorophyll and water content. Afr J Agric Res5:416-423.

Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J of Exp Bot53:1331-1341.

Andre CM, Larondelle Y, Evers D (2010) Dietary antioxidants and oxidative stress from a human and plant perspective: A review. Cur Nutri and Food Sci.62-12.

Arleta M, Wieslawa J, Barbara T (2001) Antioxidative defence to lead stress in subcellular compartment of pea root cells. Acta Bioch Polon 48:687–9.

Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant physiology*, *24*(1), 1.

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant MolBiol9:208-218.

Aslam R, Bhat TM, Choudhary, Ansari MYK, Shahwar D (2017) Estimation of genetic variability, mutagenic effectiveness and efficiency in M2 flower mutant lines of *Capsicum annuum* L. treated with caffeine and their analysis through RAPD markers. J of King Saud Uni-Sci, 29:274-283

Balestrasse KB, Benavides MP, Gallego SM, Tomaro ML (2003) Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants Func. Plant Biol, 30:57-64.

Barulina H (1930) Lentils of the USSR and other countries. Bulle of App Bot, Gen and Plant Bree, 40:265–304.

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant and Soil 39:205–7.

Bavi K, Kholdebarin B, Moradshahi A (2011) Effect of cadmium on growth, protein content and peroxidase activity in pea plants. Pak. J. Bot, 43:1467-1470.

Bhat TA, Parveen S, Khan AH (2007) Meiotic studies in two varieties of *Vicia faba* L. (Fabaceae) after EMS treatment Asian J Plant Sci. 6:51–55.

Chaudhary J, Deshmukh R, Sonah H (2019) Mutagenesis Approaches and Their Role in Crop Improvement. Plants (Basel). Oct 31;8(11):467.

Chen C, Tao C, Peng H, Ding Y (2007) Genetic analysis of salt stress responses in asparagus bean (*Vigna unguiculata* L. ssp. *Sesquipedalis verdc.*) J. Hered. 98:655–665.

Choudhary S, Ansari MYK, Khan Z, Gupta H (2012) Cytotoxic action of lead nitrate on cytomorphology of *Trigonella foenum-graecum* L. Turkish J Biol. 36:267–273.

Das S, Roy SK, (1989) Radio cytogenetical studies on *Solanum* I. meiotic abnormalities. Cytologia 54:477–481.

Daud MK, Sun Y, Dawood M, Hayat Y, Variath MT, Wu YX, Zhu S (2009). Cadmium-induced functional and ultra structural alterations in roots of two transgenic cotton cultivars. J. Hazard. Mater. 161: 463-473.

Debnath S. Chandel RK. Devi K. Khan Z (2021) Mechanism and Molecular Response of Induced Genotoxicity and Oxidative Stress in Plants. In Induced Genotoxicity and Oxidative Stress in Plants; Khan, Z., Ansari, M.Y.K., Shahwar, D., Eds.; Springer: Singapore, 2021.

Dhir B, Sharmila P, Saradhi PP (2004) Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. AquTox 66: 141–7

Dixit V, Pandy V, Shyam R (2001) Differential antioxidative responses to cadmium in roots and leaves of pea. J of Exp Bot 52:1101–9.

Duncan DB (1955) Multiple range and multiple 'F' tests. Biometrics.,11:1, 1-42.

Elloumi N, Ben F, Rhouma A, Ben B, Mezghani I, Boukhris M (2007) Cadmium induced growth inhibition and alteration of biochemical parameters in almond seedlings grown in solution culture. Acta Physio Plant 29:57–62.

Farooqi ZR, Iqbal ZM, Kabir M, NadShafiq M (2009) Toxic effect of lead and cadmium on germination and seedling growth of *Albizia lebbeck* (L.) Benth. Pakistan J Bot. 41:27–33.

Ferguson ME, Maxted N, Slageren MV, Robertson LD (2000) A reassessment of the taxonomy of Lens Mill (Leguminosae, Papilionoideae, Vicieae). Botanical J of the Linn Soc 133:41-59.

Kumar,G. Singh S. (2020) Induced cytomictic crosstalk behaviour among micro-meiocytes of *Cyamopsis tetragonoloba* (L.) Taub. (cluster bean): Reasons and repercussions. Caryologia 73(2): 111-119

Gallego, S. M., Benavides, M. P., & Tomaro, M. L. (1996). Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Science*, *121*(2), 151-159.

Girija M, Gnanamurthy S, Dhanavel D (2013) Genetic diversity analysis of cowpea mutant (*Vigna unguiculata* (L.) Walp) as revealed by RAPD marker. Int J Adv Res 1:139–147.

Gulfishan M, Khan AH, Jafri IF, Bhat TA (2012) Assessment of mutagenicity induced by MMS and DES in *Capsicum annuum* L. Saudi j of bio sci 19:251-255

Haider FU, Liqun C, Coulter JA, Cheema SA, Wu J, Zhang R, Wenjun M, Farooq M (2021) Cadmium toxicity in plants: Impacts and remediation strategies. Ecotoxicol Environ Saf. 211:111887.

Hodges DM, Long JMD, Forney CF, Prange RK (1999) Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207:604–11

Jafri IF, Khan AH, Gulfishan M (2011) Genotoxic effects of 5-bromouracil on cyto-morphological characters of *Cichorium intybus*L. African J. Biotech 10:10595-10599

Jayabalan N, Rao GR (1987). Gamma radiation induced cytological abnormalities in *Lycopersicon esculentum* Mill. var. Pusa Ruby. Cyto (Tokyo) 52:1–4

Jiang W, Liu D, Hou W (2001). Hyperaccumulation of cadmium by roots, bulbs and shoots of garlic (*Allium sativum* L.). Bioresou Techno 76:9–13.

Junaid A, Mujib A, Sharma MP (2008) Effect of growth regulators and ethyl methane sulphonate on growth, and chlorophyll, sugar and proline contents in *Dracaena sanderiana* cultured in vitro. Biol Plant 52:569–572.

Kabarity AA. El-Bayoumi, Habib A (1974) Effect of morphine sulphate on mitosis of *Allium cepa* L. root tips. Biol. Plant 16: 275-282.

Kabir M, Iqbal MZ, Shafigh M, Faroogi ZR (2008) Reduction in germination and seedling growth of *Thespesia populnea* L. caused by lead and cadmium treatments. Pakistan J Bot 40:2419–2426

Kar M, Mishra D (1976). Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Plant Physiol., 57, 315–319

Khan Z, Ansari MYK and Gupta H (2012). Induction of mutations by base analogue 6-AP (6-Amino Purine) and their detection with RAPD analysis. African Journal Of Biotechnology.11 (56):11901-11906

Khan Z, Gupta H, Ansari MYK, Chaudhary S (2009) Methyl methanesulphonate induced chromosomal variations in a medicinal plant Cichorium intybus L. during microsporogenesis Biology and Medicine, 1(2): 66-69, 2009.

Khan, Z, Shahwar, D, Ansari, M. K. Y, Chandel, R. (2019) Toxicity assessment of anatase (TiO_2) nanoparticles: A pilot study on stress response alterations and DNA damage studies in *Lens culinaris* Medik. Heliyon, 5(7), e02069.

Kumar G, Singh V (2003) Comparative analysis of meiotic abnormalities induced by gamma rays and EMS in Barley. J Indian Bot Soc. 82:19–22.

Kumar G, Srivastava S (2006) Buffering action of essential metals against heavy metal cytotoxicity in *Helianthus annus*. The Nucleus. 49:45-51

Kumar G, Tripathi R (2007) Lead-induced cytotoxicity

and mutagenicity in grass pea. Turk J of Bio, 3:73-78

Kumar G, Yadav RS (2010) EMS induced genomic disorders in sesame (*Sesamum indicum*L.) Rom. J. Biol. – Plant Biol. 55:97–104

Kumar S, Pandey G. (2020) Bio fortification of pulses and legumes to enhance nutrition. Heliyon 6 (3), e03682.

Lee G, Carrow RN, Duncan RR (2004) Photosynthetic responses to salinity stress of halophytic seashore paspalum ecotypes. Plant Sci. 166:1417–1425.

Liu D, Jiang W, Zhao FM, Lu C (1994) Effects of lead on root growth, cell division and nucleolus of *Allium cepa*. Env Poll 86:1-4

Liu JG, Li JK, Xu KQ, Zhang ZJ, Ma TB, Lu XL, Yang JH, Zhu QS (2003) Lead toxicity uptake and translocation in different rice cultivars. Plant Sci 165:793–802

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent J BiolChem 193:265–275

Maggaio A, Miyazaki S, Veronese P, Fujita T, Ibeas JI, Damsz B, Narasimhan ML, Hasegawa PM, Joly RJ, Bressan RA (2002) Does proline accumulation play an active role in stress induced growth reduction? Plant J.31:699-712

Malecka A, Jarmuszkiewicz W, Tomaszevska B (2001) Antioxidative defense of lead stress in subcellular compartments of pea root cells. Acta Biochim Polon 48:687–98.

Maria de Fátima de Souza Guilherme, Habyhabanne Maia de Oliveira and Edevaldo da Silva (2015) Cadmium toxicity on seed germination and seedling growth of wheat *Triticum aestivum*. Acta Scientiarum 37: 499-504

Meherchandani M (1975) Effect of gamma radiation on dormant seeds of *Avena sativa* L. Rad Bot 15:439–445.

Mishra RK, Singhal GS (1992) Function of photosynthetic apparatus in intact wheat leaves under highlight and heat stress and its relationship with peroxidation of thylakoid lipids. Plant Physio 98:1–6

Moreno JL, Hernandez T, Garcia C (1999) Effects of cadmium containing sewage sludgecompost on dynamics of organic matter and microbial activity in an arid soil. Biol and Fertility of Soils 2: 8230–7

Nanjo T, Kobayashi M, Yoshiba Y, Sanada Y, Wada K, Tsukaya H, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (1999) Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic *Arabidopsis thaliana*. Plant J. 18:185–192

Sharma N., Shahwar D. Choudhary S (2022). Induction of chromosomal and morphological amelioration in lentil (*Lens culinaris* Medik.) mutagenized population developed through chemical mutagenesis. *Vegetos*, 35:474–483.

Pandit BR, Prasannakumar PG (1999) Effect of metals on jowar (*Sorghum bicolor* L.) seedling growth, germination and absorption of elements. Pollu Res 18:307–15.

Patra M, Bhowmik N, Bandopadhyay B, Sharma A (2004) Comparison of mercury, lead andarsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Env and Exp Bot 52:199–223

Pavadai P, Dhanavel D (2004) Effect of EMS, DES and colchicines treatment in soybean. Crop Res. 28:118–120

PetrescuI, Sarac I, Bonciu E, Madosa E, RosculeteCA, Butnariu M (2020). Study regarding the cytotoxic potential of cadmium and zinc in meristematic tissues of basil (*Ocimumbasilicum* L.). Caryologia 73(1): 75-81. doi: 10.13128/caryologia-138

Yu, Q., &Rengel, Z. (1999). Micronutrient deficiency influences plant growth and activities of superoxide dismutases in narrow-leafed lupins. *Annals of Botany*, *83*(2), 175-182.

Qian H, Li L, Sun W, Chen GD, Sheng W, Liu, Fu Z (2009) Combined effect of copper and cadmium on *Chlorella vulgaris* growth and photosynthesis-related gene transcription. Aqua Toxico 9:456–61

Rathore H, Punyasi R, Joshi P, Rathore D, Bhatnagar D (2007) Studies on the reversal of lead induced mitostatic effect in *Allium cepa* root tip cells with myrobalan (fruit of *Terminalia chebula*, Retz, Combretaceae). Internet J Altern Med 4(1)

Ruan C, Lian Y, Lium J (1992) Application of micronucleus test in *Vicia faba* root tips in the rapid detection of mutagenic environmental pollutants. Chinese J Environ Sci. 4:56–58

Ruley AT, Sharma NC, Sahi SV (2004) Antioxidant defense in a lead accumulating plant, *Sesbania drummondii*. Plant Physio and Biochem42:899-906

Salama ZA, El-Beltagi HS, El-Hariri DM (2009) Effect of Fe deficiency on antioxidant system in leaves of three flax cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37:122–8.

Saradhi PP, Alia V B, (1993). Inhibition of mitochondrial electron transport is the prime cause behind proline accumulation during mineral deficiency in *Oryza sativa*. Plant Soil 155/156: 465 8.

Shafiq M, Zafar MI, Athar M (2008) Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala* . J. Appl. Sci. Environ. Manage 12:61–66.

Shahwar D, Ansari MYK, Bhat TM (2016) Assessment of genetic variability, morphology and productivity response of *Vicia faba* under the stress of lead nitrate, International J of Adv Life Sci9:58-64.

Shahwar D, Ansari MYK, Choudhary S (2018) Evaluation of Genotoxic Potential of Heavy Metal in a Proteina-

ceous Crop (*Lens culinaris* Medik) in Aspect to Cytomorphological Parameters.J of Bio Sci 18:208-215.

Shahwar, D., Ansari, M. Y. K., Bhat, T. M., Choudhary, S., & Aslam, R. (2017). Evaluation of high yielding mutant of lentil developed through caffeine of an exotic germplasm. *Int. J. Plant Breed. Genet*, *11*, 55-62.

Shahwar, D., Ansari, M. Y. K., Choudhary, S. (2019). Induction of phenotypic diversity in mutagenized population of lentil (*Lens culinaris* Medik) by using heavy metal. *Heliyon*, *5*(5), e01722.

Shahwar, D., Ansari, M. Y. K., Choudhary, S., Aslam, R. (2017). Evaluation of yield attributing variants developed through ethyl methane sulphonate in an important proteinaceous crop-*Vicia faba*. *Asian J. Crop Sci*, *9*, 20-27.

Shahwar, D., Khan, Z., Ansari, M. Y. K. (2020). Evaluation of mutagenized lentil populations by caffeine and EMS for exploration of agronomic traits and mutant phenotyping. *Ecological Genetics and Genomics*, *14*, 100049

Shahwar, D., Ansari, M. Y. K., & Park, Y. (2022). Physiobiochemical analysis and molecular characterization of induced lentil mutant lines. *Plos one*, *17*(10), e0274937.

Shehab GG, Ahmed OK, El-Beltagi HS (2010) Effect of various chemical agents for alleviation of drought stress in rice plants (*Oryza sativa* L.) Notulae Botan Horti Agrobotanici Cluj-Napoca 38:139–48.

Siler B, Misic D, Filipovic B, Popovic Z, Cvetic T, Mijovic A (2007). Effects of salinity on in vitro growth and photosynthesis of common centaury (*Centaurium erythraea* Rafn.). Arch. Biol. Sci. 59:129–134.

Singh M, Khanna VK, (1988) Effect of gamma radiation on the crossability of wheat, triticale and rye on meiosis: pollen grains germination and pollen tube growth. Cyto 53:123–130.

Sinha R, Pal AK, Singh AK (2018) Physiological, biochemical and molecular responses of lentil (*Lens culinaris* Medik.) genotypes under drought stress, Ind J Plant Physiol. 23:772-784

Smith J H, Benitez A (1955) Chlorophylls: analysis in plant materials. In Modern Methods of Plant Analysis/Moderne Methoden der Pflanzenanalyse Springer Berlin Heidelberg, 142-196

Srivastava A, Kapoor K (2008) Seed yield is not impaired by chromosome stickiness in sodium azide treated *Trigonella foenum-graecum*. Cyto 73:115-121

Sundaravadivelu K, Ranjithselvi P, Reddy VRK (2006) Induced genetic variability in cotton *(Gossypium hirsutum* L.*)* for yield and its components. Crop Res 32: 442–446

Suprasanna P, Mirajkar SJ, Bhagwat SG (2015) Induced mutations and crop improvement. In Plant bio and biotech, vol I. plant diversity organization function and improvement Springer, New Delhi, 593-617

Şuţan NA. Blănaru RG, Şuţan C (2018) Cytogenotoxic potential of surface water – a case study of Argeş River, Romania. Current Trends in Natural Sciences, 7(13):312-318.

Suzuki N (2005) Alleviation by calcium of cadmium-induced root growth inhibition in *Arabidopsis* seedlings. Plant Biotech 22:19–25

Tharanathan RN, Mahadevamma S (2003) Legumes – A boon to human nutrition. Trends in Food Science and Technology, 14:507–518

Thilagavathi C, Mullainathan L (2011) influence of physical and chemical mutagens on quantitative characters of *Vigna mungo* (L.Hepper) IRMJ-Agriculture,1/1:06-08.

TripathiR, Kumar G (2010) Genetic loss through heavy metal induced chromosomal stickiness in Grass pea, Caryologia, 63:3, 223-228.

Tripathy BC, Oelmüller R (2012) Reactive oxygen species generation and signaling in plants. Plant Signal Behav, 7(12):1621-33.

Unyayar S, Celik A, Cekic FO, Gozel A (2006) Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. Mutagenesis 21:77–81.

Utsunomiya KS, Bione NCP, Pagliarini MS (2002) How many different kinds of abnormalities could be found in unique endogamous maize plant? Cyto 67:69-176

Van AF, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environment 13:195–206

Van OSS H, Amn Y, Ladizinsky G (1997) Chloroplast DNA variation and evolution in the genus lens Mill. 'Theo and Applied Gen 94: 452-457.

Verma AK, Singh RR, Singh S (2012) Cytogenetic effect of EMS on root meristem cells of *Catharanthusroseus* (L.) G. Don var. Nirmal. Inter J Pharm BiolSci 2:20–24.

Wong JWC, Wong MH (1990) Effects of fly ash on yields and elemental composition of two vegetables, *Brassica parachinensis* and *B. chinensis*. Agric Ecosyst Environ 30:251–264

Xu BJ, Chang SKC (2010) Phenolic substance characterization and chemical and cell-based antioxidant activities of 11 lentils grown in the northern United States. J. Agric. Food Chem 58: 1509–1517

Yi H, Meng Z (2003) Genotoxicity of hydrated sulphur dioxide on root tips of *Allium sativum* and *Vicia-faba*. Mutat Res 537: 109-114

Zengin FK, Munzuroglu O (2006) Toxic effects of cadmium (Cdþþ) on metabolism of sunflower (*Helianthus annuus* L.) seedlings. Acta Agricul Scand, Section B–Soil & Plant Science 56: 224–9.