



# Induction of phenotypic diversity in mutagenized population of lentil (*Lens culinaris* Medik) by using heavy metal



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## ABSTRACT

Pulse breeding has been performed in the past by utilizing the genetic variability using conventional method. At the present time, these techniques are insufficient for producing new cultivars to fulfill globally increased food demand. In this situation, induced mutagenesis have been appeared as a new technique which are largely utilized for evolving improved mutants with good quality of agronomic traits and for determining desired genes that control agronomical traits. In the present investigation lentil seeds were mutagenized with different doses (5, 10, 15, 20 and 25 ppm) of lead and cadmium nitrate. M<sub>2</sub> generation was raised from collected seeds of M<sub>1</sub> generation. Distinct morphological mutants were selected with different traits such as plant height, growth habit, leaf morphology, flower character, pigmentation and pod size. Different meiotic aberration such as stickiness, precocious separation of chromosome, unequal division, disturbed polarity with laggards, cytotoxicity, disorientation, unpolarized chromosome, sticky metaphase, multinucleate condition with micronuclei were also observed in this experiment. Some mutants may be utilized directly in selection or some of these are beneficial in breeding programme. Beneficial mutants were determined at lower concentrations both heavy metals with highest mutation frequency in cadmium than lead nitrate.

## 1. Introduction

Pulse crops belong to the fabaceae family known to be annually cultivated leguminous crops sown for their seeds. Pulses are utmost important agricultural product which is grown in most of countries of world. It is important source of proteins as well as carbohydrates, dietary fibers, essential macro and micro nutrients like vitamins and minerals. Pulse crops play an important role in the diets of poor people across the globe because of high amount of proteins, vitamins and minerals. It is valuable protein-rich food and animal feed grown by human being in developing countries (Zong et al., 2009). The mutagenic treatments induce mutations which affects plant height, branching and leaf morphology in lentil (Bhat et al., 2006). The present experiment was performed to investigate the effects of heavy metals (lead and cadmium) on *Lens culinaris* for the induction of mutations and useful variations. *Lens culinaris* is a small genus of legume that includes the cultivated lentil (*Lens culinaris* Medik subsp. *culinaris*) and 6 associated taxa (Ferguson et al., 2000). Lentil is bushy, annual, self pollinated, diploid ( $2x = 2n = 14$ ) food crop. Lentil is also referred as one of the nutritious legume crop after the chickpea amongst the rabi (winter) crops. It is generally grown during rabi season (October to April). It has been originated in East

Mediterranean region, such as Asia Minor and Egypt. India is third producer of lentil. It was reported that only 5% of the total area of pulses cultivation is shared by lentil. The major lentil producing states of India are Madhya Pradesh, Uttar Pradesh, Bihar, Uttarakhand and West Bengal.

Heavy metals naturally present in environment. In Modern civilization, it is considered that heavy metals produced by different anthropogenic activities such as factories, transportation and by using agricultural pesticide and fertilizers which cause pollution to the atmosphere. Excessive levels of heavy metals are dangerous to plants, animals and human health (Järup, 2003; Azevedo and Lea, 2005). Presently, contamination of soil in cultivated fields containing harmful heavy metals such as cadmium, lead, copper, nickel and zinc has appeared as a new threat to agriculture (Singh et al., 2007) and hazardous health problems. Many studies have been performed by researchers to evaluate the effects of heavy metals on plants (Baker et al., 2000). It was reported by several workers that cadmium is genotoxic for plant (Kumar and Rai, 2007; Kumar and Tripathi, 2007), and it also shows carcinogenic and mutagenic potential to plant due to exposure of higher doses of lead and cadmium and disrupting cell division and root growth in lentil (Kiran and Ahmad, 2006), *Allium cepa* (Liu and Kottke, 2004), *Zea mays* L. (Jiang and

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Liu, 2000), *Helianthus annuus* (Kumar and Srivastava, 2006), *Allium sativum* (Yi and Meng, 2003). Several researchers observed that seed germination and plant development is decreased by cadmium (Aydinalp and Marinova; 2009; Khan and Sahin, 2006) and also cause chlorophyll mutation at higher concentration.

Lead and cadmium were used as a mutagen in the present investigation and it was selected on the basis of mode of actions in lentil. Lead is the most dangerous polluting agent for human, animals and plants. It binds strongly to a large number of molecules of nucleic acid like DNA and RNA. DNA synthesis is changed by heavy metals which causes mitotic activity. Depolymerisation appears due to variability in genome which causes abnormal nitrogenous bases, breakage in double stranded DNA, DNA-DNA cross-links and DNA – protein cross-link. It was demonstrated that abnormal bases i.e thymine glycol and 8- hydroxy-guanine are produced due to DNA damage through a sequence of reactions which started by the abstraction of 4'- hydrogen atom of a ribose sugar. Reddy et al. (2005) reported that lead reacts with sulfhydryl groups of enzymes and inhibits enzyme's activity which may produces oxidative stress by enhancing the generation of ROS (Reactive oxygen species) in pants. It was observed that reduce uptake of mineral elements inhibits the different type of enzymes activities and induction of oxidative stress caused by excessive amount of Cd (Sandalio et al., 2001).

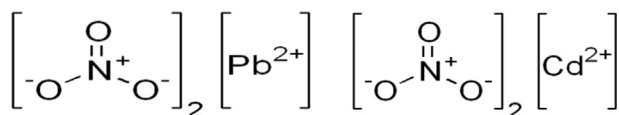


Fig. Structure of Lead nitrate and Cadmium nitrate.

The aim of the current study is inducing phenotypic variations and evaluation of morphological mutants in lentil by using different doses of heavy metals like Pb and Cd. This evaluated mutant may be helpful to produced improve variety with modifying traits or characters through cross breeding programme.

## 2. Material and methods

### 2.1. Experimental plant and procedure

#### 2.1.1. Experimental plant

Healthy, fresh and certified seeds of (*Lens culinaris Medik*) var, L – 4076 were collected from Indian Agriculture Research Institute IARI, Pusa campus, New Delhi India.

#### 2.1.2. Mutagen used

In the present examination, lead nitrate and cadmium nitrate were used as mutagen. Seeds were treated with different concentration of lead nitrate and cadmium nitrate as detailed below:

### 2.2. Heavy metal (lead nitrate and cadmium nitrate) treatment

For mutagenic treatment, lentil seeds were presoaked in distilled water for overnight and then treated with five different doses (5, 10, 15, 20 and 25) of lead nitrate and cadmium nitrate solution for 12 hrs with continuous shaking at room temperature  $25 \pm 2$  °C. Seeds were washed thoroughly after treatment under running tap water for 30 min to remove adhered mutagen's particle on the surface of seed coat. Extensively washed 100 seeds of each treatment were sown in pot of five replicate along with control to raise M<sub>1</sub> generation. The experiment was performed during rabi season (winter) season of 2015–2016 in Aligarh. The experimental site has characteristics semi-arid and sub-tropical climate with hot dry summers and cold winters. The average rainfall is 847.30 mm, while the average temperature is 35 °C and 15 °C during summer and winter, respectively. The soil of Aligarh is sandy loam and alkaline. Agricultural practices were performed for the sowing and subsequent management of the lentil crop.

To raise M<sub>2</sub> generation, twenty five (25) seed from M<sub>1</sub> generation of each treatment were sown along with their control in three replicates during winter season of 2016–17. Control as well as treated plant had 50 progenies (Table 1). Growing condition of M<sub>2</sub> plant is same as growing condition of M<sub>1</sub> plant.

### 2.3. Mutation frequency

Mutation frequency of variants/mutants was calculated using following formula-

$$\text{Variation/Mutation frequency (\%)} = \frac{\text{No. of plants with mutant characters}}{\text{Total number of plants survived}} \times 100$$

### 2.4. Meiotic studies

To perform cytological studies, young flower buds from selected mutant plants as well as control, were fixed in freshly made Carnoy's fixative for 24 h and stored in 70% alcohol. Anthers from the collected flower buds were squashed in 1% propionocarmine and prepared permanent slide through NBA-GAA series for pollen mother cells and pollen grain analysis.

## 3. Results

Morphological variants/mutants were selected attributing various agronomic traits in lentil such as plant height, growth habit, leaf morphology, pigmentation (anthocyanin), flower characters and pods.

### 3.1. Bifoliate leaves

Bifoliate leaflets were observed in 5 ppm Lead nitrate and 15 ppm Cadmium nitrate (Plate 1, A). Mutation frequency of bifoliate leaves was recorded 2.4% in lead nitrate whereas 3.2 in cadmium nitrate. Percentage of total frequency was 5.6 % (Table 2) in *Lens culinaris*.

### 3.2. Two unequal size leaflets

Two unequal size leaflets, one oblong, obovate, entire and large another one very small was observed (Plate 1, B). They occurred in the moderate doses of lead and cadmium nitrate. Mutation frequency of unequal size leaflets were 2.0% and 2.4% in lead and cadmium nitrate respectively. Total frequency of this mutant was 4.4% (Table 2).

### 3.3. Bilobed leaflet

Bilobed leaflet possessed two lobes, slightly notched at lateral side

Table 1

Number of plants in M<sub>2</sub> on which the observation of frequency of morphological mutation was carried out in the *Lens culinaris Medik* (variety L-4076).

| Treatment                               | No of M <sub>1</sub> plant progenies | No. of plant progenies segregating in M <sub>2</sub> | % Mutated plant progenies (Mp) | No. of M <sub>2</sub> seedling |
|---|--------------------------------------|--|--------------------------------|--------------------------------|
| Control                                 | 50                                   | –  | –                              | 1248                           |
| Pb(NO <sub>3</sub> ) <sub>2</sub> (ppm) |                                      |  |                                |                                |
| 5                                       | 50                                   | 2  | 4                              | 1200                           |
| 10                                      | 50                                   | 4  | 8                              | 1188                           |
| 15                                      | 50                                   | 6  | 12                             | 1154                           |
| 20                                      | 50                                   | 7  | 14                             | 1125                           |
| 25                                      | 50                                   | 9  | 18                             | 1100                           |
| Cd(NO <sub>3</sub> ) <sub>2</sub> (ppm) |                                      |  |                                |                                |
| 5                                       | 50                                   | 3  | 6                              | 1190                           |
| 10                                      | 50                                   | 5  | 10                             | 1176                           |
| 15                                      | 50                                   | 6  | 12                             | 1147                           |
| 20                                      | 50                                   | 8  | 16                             | 1115                           |
| 25                                      | 50                                   | 10   | 20                             | 1085                           |



**Plate 1.** Induced phenotypic diversity of mutagenized population of *Lens culinaris*, A: bifoliate, oblong, vegetative leaf, B: two unequal size leaf one is large another is small, C: bilobed (unequal) thick, apical leaf, D: opposite leaf, E: heart shaped leaf, F: trifoliate, oblong shape with entire margin leaf, G: trilobed, H: chlorophyll mutant, I: tetrapartite leaflet, J: bushy mutant, K: one sided branch mutant L: curl leaf mutant M: dwarf mutant N: single branch mutant, O: tall mutant, P: mutant with deposition of anthocyanin, Q: three flower, R: single flower, S: small pod, T: medium and bold pod, U: large pod, V: very large and bold pod.

(Plate 1, C). Mutant bearing bilobed leaflet had large leaflets, pods and seeds. It obtained at 25 ppm lead and 15, 20 ppm cadmium nitrate. Mutagenic frequency of bilobed leaflet was 3.6 % and 3.2 % in lead and cadmium nitrate respectively. Total frequency of this mutant was recorded 6.8 % (Table 2).

### 3.4. Opposite leaflets

Mutants bearing broad, oval opposite leaflets differ with control in which leaflets were arranged in alternate fashion (Plate 1, D). Mutant with opposite leaflets possessed normal growth and increase number of pods and seeds was recorded only 10 ppm lead nitrate. Frequency of such mutants was 1.6% (Table 2).

### 3.5. Heart shape leaflet

Heart shape leaflet was recorded at lower concentration of both mutagens. It exhibited broad, thick, large, obcordate, obovate, entire, green leaflet on rachis. These mutants showed higher number of pods and bold seed (Plate 1, E). Mutation frequency of such mutant was reported 1.2% and 0.8% in lead and cadmium nitrate respectively. Total frequency of these mutants was found to be 2.0% (Table 2).

### 3.6. Trifoliate leaves

Trifoliate leaves were noticed at 5 ppm lead and 15 ppm cadmium nitrate. Mutant having trifoliate leaves exhibited different characters in leaflets such as large, elongated, oblong and entire but slightly curved upward (Plate 1, F). They also possessed fertile branches and moderate yield. Frequency of trifoliate leaves was 2.8% in lead nitrate and 3.6% in cadmium nitrate. Total frequency of trifoliate leaves was 6.4% (Table 2).

### 3.7. Tri-lobed leaflet

Trilobed leaflet exhibited notches at three points. Mutant with trilobed leaflet had thick and larger size leaves, pods and seeds (Plate 1, G). These mutants were screened at higher and lower doses of lead and cadmium nitrate. Mutation frequency of tri-lobed leaflets was lower in comparison to bilobed leaflet. Frequency of this mutant was 1.2% in lead nitrate and 2.0% in cadmium nitrate. Total mutation frequency of trilobed leaflet was 3.2% (Table 2).

### 3.8. Chlorophyll mutants

Chlorophyll mutant was observed at lower concentration of both mutagens. In this mutant one branch exhibiting xantha type chlorophyll mutation and all leaflets in other branches varied in shape and size (Plate 1, H). 1.6% and 2.4% mutation frequency was recorded for chlorophyll

mutants in lead and cadmium nitrate respectively. Total frequency of this mutant was found to be 4.0% (Table 2).

### 3.9. Tetrapartite leaves

Tetrapartite leaves with entire margin but curved upwards and obtuse apex was obtained at 20 ppm cadmium nitrate (Plate 1, I). Mutant having such type of leaves exhibited vigorous growth, low fertility and yield. Total mutation frequency of tetrapartite leaves was 0.8% (Table 2).

### 3.10. Bushy mutants

Bushy mutants showed increase number of branches and leaves. Because of increase of number of branches it shows bushy appearance (Plate 1, J). Increase number of branches produced increase number of pods, so that yield was also increase. Bushy mutants were observed at lower concentration of lead and cadmium nitrate. Mutation frequency of bushy mutants was 0.8% in lead nitrate while 2.0% in cadmium nitrate (Table 2).

### 3.11. One sided branch mutants

One sided branch mutant were screened and isolated at 20 ppm lead nitrate. In this plant, lateral branches were found on one side of stems. Branches were developed normally on both side in the beginning but later they fell down from one side. This type of mutant produced moderate number of pods, but the pod and seed size was bigger (Plate 1, K). Total mutation frequency of one sided branch mutant was 0.4% (Table 2).

### 3.12. Curl leaf mutant

Curl leaf mutant was noticed at 5 ppm cadmium nitrate. It exhibited short height and curling of leaf. This type of mutants was shown late flowering and maturity having less number of pods and small size seeds (Plate 1, L). Total frequency as against the total morphological mutations was 1.6 % in lentil (Table 2).

### 3.13. Dwarf mutants

Dwarf mutants were found at 10 and 5 ppm lead and cadmium nitrate respectively. It possessed short internodes with weak stem, decreased number of branches, white and purple single flower per peduncle, large bold and increase number of pods, small, smooth, reddish brown seeds and improved yield (Plate 1, M). Frequency of this mutant was higher in cadmium nitrate than lead nitrate. Total mutations frequency of dwarf mutants was 2.8 % (Table 2).

### 3.14. Single branched mutants

Single branched mutant had only one branch with more height (Plate 1, N). Decrease number of pod and yield were recorded in the single branch mutant because of less number of branches.

This type of mutant was selected at 5 and 15 ppm lead and cadmium nitrate respectively. Frequency of this mutant was 2.0 % in lead nitrate and 1.2 % in cadmium nitrate. Total frequency of this mutant was 3.2 % in lentil (Table 2).

### 3.15. Tall mutants

Tall mutants exhibited increase height, branches with elongated leaves, long internodes, bold and large pods resulting high yield (Plate 1, O). Frequency of this mutant was greater than dwarf mutants (Table 2). It was assessed at lower concentrations of lead and cadmium nitrate.

### 3.16. Anthocyanin containing mutant

Anthocyanin containing mutant was noticed at moderate concentrations of both mutagens. Leaves of this mutant had red patches resulting red color leaves (Plate 1, P). Frequency of this mutant was higher in cadmium nitrate as compared to lead nitrate. Total frequency of this mutant was 3.2 % in lentil (Table 2).

### 3.17. Three flower mutants

Some mutants selected on the basis of three flowers per peduncle instead of two flowers (Plate 1, Q). Flowers were light purple color. Three flower mutant possessed large leaflets, pods and seeds. These mutants showed normal growth with high yield. They were determined at 5 and 10, 15 ppm lead and cadmium nitrate with the frequency of 2.0 % and 3.2 % in lead cadmium nitrate respectively (Table 2).

### 3.18. Single flower mutants

Mutant having single flower was recorded at 20 ppm of both mutagens with the frequency of 0.8 % in lead nitrate and 1.2% in cadmium nitrate (Table 2). These mutants had single flower per peduncle instead of two flowers (Plate 1, R). Flowers were white in color with large sepals. These mutants showed normal growth with small pods and seeds resulting low yield.

### 3.19. Small pods

Mutants exhibiting small pods were appeared at 20 and 15, 20 ppm of lead and cadmium nitrate. Mutant plant had small, bold, light brown pod (Plate 1, S). Mutants contained narrow leaves, medium, reddish brown seeds and exhibited considerable decrease in yield. These mutants possessed normal growth with the Frequency of 2.0 % in lead nitrate and 1.6 % in cadmium nitrate (Table 2).

### 3.20. Medium and bold pods

These mutants were possessed medium, dark brown and bold pods (Plate 1, T). These mutants possessed normal growth, large seeds. Because of medium, bold pod and large seeds and considerable increase in yield were observed. It was recorded at moderate doses of both mutagens. Frequency of this mutant was higher in lead than cadmium (Table 2).

### 3.21. Large pods

Some mutants produced large, bold, light brown pods, isolated from lower concentration of lead and cadmium nitrate. Because of having large, bold pods and seeds it would considerably increase in yield over

the control (Plate 1, U). These mutants possessed normal growth. Frequency of this mutant was 3.6 % in lead nitrate and 3.2 % in cadmium nitrate (Table 2).

### 3.22. Very large and bold pods

Mutant having very large, bold pods and seeds was screened out at 5 and 10 ppm cadmium nitrate (Plate 1, V) and such type of mutants showed better growth and yield. Total frequency of these mutants was 4.0% (Table 2).

### 3.23. Cytological/meiotic studies

Cytological studies of mutant plant as well as control were done to observe mutagenic potential of heavy metal. Control plant showed normal meiotic division at metaphase I and anaphase I (control) (Plate 2, a and b) and mutant plants exhibited different meiotic lesion in PMCs. Most frequently found abnormalities were stickiness, precocious separation of one chromosome at metaphase I (Plate 2 fig c), unequal division, movement of trivalent to one pole at anaphase I (Plate 2 fig d), disturbed polarity with laggards at telophase (Plate 2 fig e), cytomixis between three cells (Plate 2 fig f), disorientation, unpolarized chromosome at anaphaseII (Plate 2 fig g), sticky metaphase I (Plate 2 fig h), multinucleate condition with micronuclei at telophase II (Plate 2 fig i) etc.

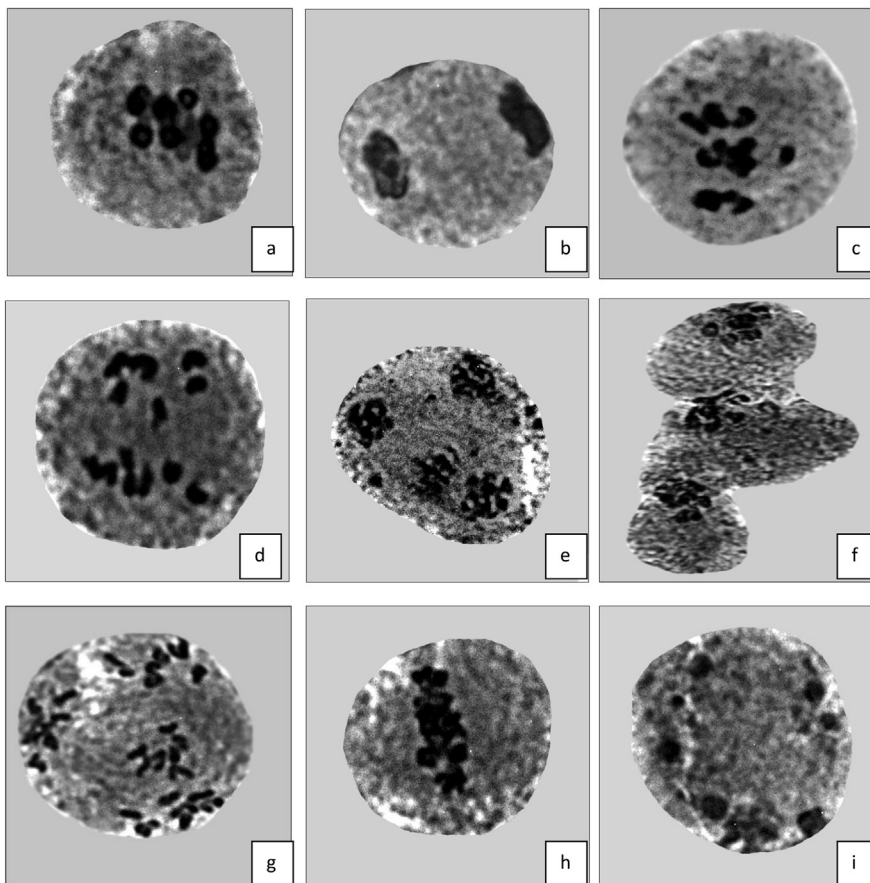
### 3.24. Frequency of morphological mutations

The frequency of morphological mutants varied with various categories of plant phenotypes in distinct mutagenic treatments (Tables 2 and 3). Mutation frequency was recorded 44.8 % in cadmium nitrate higher than frequency of lead nitrate (34.8) %. Total frequency of morphological mutants was 81.2% (Table 3). Tall, bushy, leaf and pod mutants developed more generally at lower and moderate concentrations of both the mutagen.

## 4. Discussion

In the mutation breeding programmes, morphological mutations are a desirable direction to improve different traits of crops. It has been described by several workers that macromutations (morphological mutations) are caused by chromosomal abnormalities and gene mutations (Singh et al., 1980). It was observed by Nilan (1967) that percentage of different mutant phenotypes might be result of different mutagenic treatments.

In the present study, tall mutant was observed in M<sub>2</sub> generation. It was also reported by Jana (1963) and Kumar et al. (2009) in blackgram, Solanki et al. (2004), Khurshed and Khan (2014) in lentil. Dwarf mutant were also recorded in this experiment. It exhibited small internodes which might be due to decrease in cell division. several researchers also described about dwarf plants such as Sethi (1974) in barley, Talukdar and Biswas (2006) in grass pea, Arulbalachandran and Mullainathan (2009) in blackgram, Khan et al. (2011) in chickpea, Wani et al. (2011) in *Vigna* species. It was reported by Hedens (2003) that reduction in plant height was due to alteration in gibberellic acid whereas Kleinhofs et al. (1978), Suganthi et al. (1994) reported that decrease in plant height was due to mitotic irregularities. Konzak et al. (1969) in Triticum and Shaloor et al. (1978) in triticale revealed that semi-dwarf characteristic was regulated by polygenes. In the present investigation one sided branched mutant was observed in M<sub>2</sub> generation. It was also recorded by Khan et al. (2011) in chickpea. The withering of branches on one side of stem of plants may be due to disproportion of non functioning hormone. It was suggested by the Davis and Addicott (1972) that physiological effects of abscisic acid which affects growth and development of plants. Abscisic acid also has direct relationship with incision of young fruits with senescence and dehiscence of mature fruits. Rekha et al. (2000) suggested that mutagenic treatments change activity of hormones in *Artemisia pallens*. A



**Plate 2.** Showing chromosomal aberrations in mutant plants of lentil, a: diakinesis, b: anaphase I (control), c: stickiness, d: precocious separation of one chromosome at metaphase I, e: Unequal division, movement of trivalent to one pole and bivalent to another pole at anaphase I, f: disturbed polarity with laggards at telophase II, g: cyto-mixis between three cells, h: disorientation, unpolarized chromosome at anaphase II, i: sticky metaphase I, j: Multinucleate condition with micronuclei at telophase II.

**Table 2**  
Frequency and spectrum of morphological variants/mutants induced by various mutagens in *Lens culinaris* Medik (variety L-4076).

| Mutant type             | Lead nitrate  |             |           | Cadmium nitrate |             |           | Total Freq (%) |
|-------------------------|---------------|-------------|-----------|-----------------|-------------|-----------|----------------|
|                         | No. of mutant | Conc. (ppm) | Freq. (%) | No. of mutant   | Conc. (ppm) | Freq. (%) |                |
| Plant height            |               |             |           |                 |             |           |                |
| Tall                    | 4             | 10          | 1.6       | 4               | 5           | 1.6       | 3.2            |
| Dwarf                   | 3             | 10          | 1.2       | 4               | 5           | 1.6       | 2.8            |
| Growth habit            |               |             |           |                 |             |           |                |
| Bushy                   | 2             | 15          | 0.8       | 3               | 10          | 1.2       | 2.0            |
| One sided branching     | 1             | 20          | 0.4       | –               | –           | –         | 0.4            |
| Single branched         | 5             | 10          | 2.0       | 3               | 15          | 1.2       | 3.2            |
| Curl leave mutant       | –             | –           | –         | 4               | 5           | 1.6       | 1.6            |
| Leaf                    |               |             |           |                 |             |           |                |
| Bifoliate               | 6             | 5           | 2.4       | 8               | 15          | –         | 3.2            |
| Two unequal size        | 5             | 15          | 2.0       | 6               | 10          | 2.4       | 4.4            |
| Bilobed                 | 9             | 25          | 3.6       | 8               | 20, 15      | 3.2       | 6.8            |
| Opposite                | 4             | 10          | 1.6       | –               | –           | –         | –              |
| Heart shaped            | 3             | 10          | 1.2       | 2               | 10          | 0.8       | 2.0            |
| Trifoliate              | 7             | 5           | 2.8       | 9               | 15          | 3.6       | 6.4            |
| Trilobed                | 3             | 25          | 1.2       | 5               | 10          | 2.0       | 3.2            |
| Chlorophyll             | 4             | 10,15       | 1.6       | 6               | 5, 10       | 2.4       | 4.0            |
| Tetrapartite/Tetralobed | –             | –           | –         | 2               | 20          | 0.8       | 0.8            |
| Pigmentation            |               |             |           |                 |             |           |                |
| Anthocyanin             | 3             | 15          | 1.2       | 5               | 10          | 2.0       | 3.2            |
| Flower                  |               |             |           |                 |             |           |                |
| Three flowers           | 5             | 15          | 2.0       | 8               | 10, 15      | 3.2       | 5.2            |
| Single flowers          | 2             | 20          | 0.8       | 3               | 20          | 1.2       | 2.0            |
| Pod                     |               |             |           |                 |             |           |                |
| Small pod               | 5             | 20          | 2.0       | 4               | 20, 15      | 1.6       | 3.6            |
| Medium pod              | 7             | 15          | 2.8       | 6               | 10          | 2.4       | 5.2            |
| Large pod               | 9             | 10          | 3.6       | 8               | 5           | 3.2       | 6.8            |
| Very large pod          | –             | –           | –         | 5               | 10, 5       | 4.0       | 4.0            |

combined study of molecular genetics and physiology was performed by Shimizu-Sato and Mori (2001) and observed that branching pattern of

**Table 3**Total pooled frequency and spectrum of different types of morphological variants/mutants induced in *Lens culinaris* Medik variety L-4076.

| Treatment       | Morphological mutant types (%) |              |      |              |        |      | Total frequency (%) |
|-----------------|--------------------------------|--------------|------|--------------|--------|------|---------------------|
|                 | Plant height                   | Growth habit | Leaf | Pigmentation | Flower | Pod  |                     |
| Mutagen basis   |                                |              |      |              |        |      |                     |
| Lead nitrate    | 2.8                            | 3.2          | 16.4 | 1.2          | 2.8    | 8.4  | 34.8                |
| Cadmium nitrate | 3.2                            | 7.2          | 18.4 | 2.0          | 4.4    | 11.2 | 44.8                |
| Total           | 6.0                            | 10.4         | 34.8 | 3.2          | 7.2    | 19.6 | 81.2                |

plant was determined by growth hormone as well as gene involved in organ formation. So that, more number of branches produced due to loss of apical dominance resulting lateral distribution of growth hormone and increasing number of branching leads to bushy appearance. Tall mutant with more branches were recorded by EMS treated population in cowpea by Gnanamurthy and Dhanavel (2014). Naik et al. (2002) observed bushy plant with narrow leaflets increasing the yield because sunlight reached all parts of plants specially stem and leaflets which increases the photosynthetic efficiency. Satyanarayana et al. (1989), Talukdar (2009) determined that morphological mutants such as dwarf, tall and bushy are monogenic recessive. In this study, chlorophyll mutant were also found. Development of chlorophyll found to be managing by several genes situated on different chromosomes which could be adjoining to centromere and proximal segment of chromosomes (Swaminathan, 1964). Different types of mutants were examined by Wani and Anis (2004), Arulbalachandran and Mullainathan (2009) in *Vigna mungo*. Morphological variants/mutants of leaf were observed in mutant plant such as color of foliage (light, medium, and dark) and leaflet size (small, medium and large), and shape of leaflet (narrow and broad). Dixit and Dubey (1983) also observed variations in length, rachis, width and arrangement of leaflet in different lentil morphological mutants. Different leaflet variations/mutations ranged from broad to small leaflets were found. Different morphological mutants have also been observed by Shah et al. (2006) in Chickpea. Single to triple flower per peduncle instead of two were observed. It was also reported by Amin et al. (2015) in lentil, Khursheed et al. (2017) in faba bean. Different flower mutants were recorded by Aslam et al. (2017) in *Capsicum annum*. Mutant plant with pod variations in terms of size (small, medium, large and very large), shape (bold) were distinguished. These types of mutants are remarkable interest since these mutants exhibited considerable improvement in plant yield. Wani and Anis (2008) observed that large pod and bold seed mutants due to result of gene mutations. Similar result was recorded by Arulbalachandran and Mullainathan (2009) in *Vigna mungo*; Shah et al. (2011) in *Cicer arietinum*; Amin et al. (2015) in *Lens culinaris*; Khursheed et al. (2017) in *Vicia faba*. In leguminous plants, easy induction of mutation may be due to the leaf abnormalities/irregularities (Blixt, 1972) or due to chromosomal aberrations (Grover and Virk, 1986).

In the present investigation, mutant plant exhibited chromosomal lesion such as stickiness, precocious separation of chromosome, unequal division, disturbed polarity with laggards, cytotoxicity, disorientation, unpolarized chromosome, sticky metaphase, multinucleate condition with micronuclei etc. Khan et al. (2009) suggested that movement of univalent or bivalent toward one pole which leads to unequal division of chromosomes or reduction of a complete set of chromosome. Siddiqui and Ansari (2005) demonstrated that formation of multivalent (trivalent) was due to abnormal pairing of chromosome such as translocation and inversion. Precocious separation occurs due to the effect of mutagens which alters the protein moiety of the nucleoprotein backbone (Kumar and Rai, 2007). It can be attributed that laggard at anaphase I/II is caused by delayed terminalization of chiasma or stickiness of chromosomal ends (Minija et al. (1999); or it may arise due to breakage or faulty spindle which leads to improper daughter nuclei and micronuclei (Singh and Chaudhary, 2005). Partial dissociation of nucleoproteins or alteration in the pattern of cyto-chemically balanced reactions is the reason for stickiness (Jayabalan and Rao, 1987). Disturbed polarity occurred at anaphase and telophase stages caused by disruptions in spindle fiber

resulting multinucleate condition. Similar observations were also examined in different plants by various workers such as Sharma et al. (2009), Aslam et al. (2012, 2017), Choudhary et al. (2012), Shahwar et al. (2016, 2017a, b, 2018), Amin et al. (2019) etc.

## 5. Conclusion

This investigation was undertaken to demonstrate frequency of morphological mutants of lentil treated with lead and cadmium nitrate. Different morphological variants/mutants were selected with different traits such plant height (tall, dwarf), growth habit (bushy, one sided branching, single branched, curl leaf) leaf morphology (bifoliate leaf, two unequal size leaf one is large another is small, bilobed leaf, opposite leaf, heart shaped leaf, trifoliate leaf, trilobed, chlorophyll containing leaf, tetrapartite leaf), flower character (variation in number of flower), pigmentation (anthocyanin) and pod (small, medium large very large). Most of the useful mutations were observed at lower concentration of lead and cadmium nitrate. The highest mutation frequency was found in the cadmium nitrate treated plants than lead nitrate.

## Declarations

### Author contribution statement

Durre Shahwar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M.Y.K. Ansari: Conceived and designed the experiments.

Sana Choudhary: Analyzed and interpreted the data.

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### Additional information

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