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RESEARCH ARTICLES

Induction of chromosomal and morphological amelioration in lentil (*Lens culinaris* Medik.) mutagenized population developed through chemical mutagenesis

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Abstract
Lentil (*Lens culinaris* Medik.) is an edible pulse crop with an excellent proportion of high-quality protein. The production of lentil should increase worldwide because of its increasing demand and significant economic value. In this present investigation, caffeine and Ethyl methane sulphonate (EMS) were used to induce morphological and cytological variations in M_1 generation of lentil. The seeds of lentil L-4076 were treated to five different concentrations (0.10, 0.25, 0.50, 0.75 and 1.0%) of caffeine and EMS to develop viable variants. Various biological parameters (germination, plant survival and pollen fertility), quantitative traits, and cytological studies were performed. These parameters get affected with increasing concentration of mutagens, and sensitivity was found higher in EMS than caffeine. Cytological studies exhibited different types of meiotic abnormalities such as stickiness, laggard, bridge, desynchronisation, disturb polarity, and micronuclei. The frequency of chromosomal aberrations was much higher in the treatment of EMS as compared to caffeine.

Keywords *Lens culinaris* · Chemical mutagenesis · Caffeine · Ethyl methane sulphonate · Cytological aberration

Introduction

Lentil (*Lens culinaris* Medik.) is an important protein crop commonly known as masur, red dhal, or split peas. It is a self-pollinated diploid (2n = 14) (Alabboud et al. 2009) rabi crop with a genome size of 4063 Mbp (Arumuganathan and Earle 1991). Despite being cheap, its seeds contain a considerable amount of dietary protein. Apart from protein, it is also rich in complex carbohydrates, dietary fiber, and essential minerals that complement the nutritional deficiencies of a cereal-based diet. Lentil cultivation enriches soil nutrients by adding nitrogen, carbon and organic matter, and also provides a much economic and monetary return to the farmers. According to FAOSTAT 2019 data, the total production of lentil in world and India were recorded approximately 5.73 and 1.23 million tonnes respectively.

Over the past decades, induced mutation technology proved to be one of the most valued approaches for broadening the narrow genetic base of lentil because of small flower size of lentil makes recombination breeding difficult. Joint FAO/IAEA Mutant Variety Database (2019) reported that the induced mutation technology has successfully created a total of 3318 mutant varieties around the world, of which 341 mutant varieties are from India. Overall, the lentil has 18 mutant varieties with only two varieties from India.

For induction of mutations, different types of mutagenic chemicals are being used. Ethyl methane sulphonate (EMS) is mono-functional alkylating agent that alkylate guanine which may pair with thymine resulting into GC to AT and AT to GC transition and thus can cause base pair deletions or insertions. Another mutagen, caffeine, has the potential to bind with photolyase resulting in inhibiting nucleotide excision repair mechanism of DNA replication (Domon et al. 1970). Caffeine also favors the formation of molecular complexes by acting as solubilizing factor resulting in various chromosomal abnormalities and changes physical properties of DNA (viz, denaturation) which results in higher rate of spontaneous mutations.

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The present study was conducted to evaluate genotypic and phenotypic variability induced by chemical mutagens (Caffeine and EMS) in *Lens culinaris* Medik.

Materials and methods

Certified seeds of *Lens culinaris* Medik. variety L-4076 were obtained from Indian Agriculture Research Institute (IARI), New Delhi, India. Fresh, healthy, and uniform seeds were pre-soaked in distilled water overnight at room temperature and were treated with different concentrations (0.1%, 0.25%, 0.5%, 0.75% and 1.0%) of caffeine and EMS solutions for 9 h. One set of seeds was soaked in distilled water to act as the control. The treated sets of seeds were thoroughly washed under running tap water to remove the excess chemicals adhering to the seed coat. To raise M_1 generation, 100 seeds were sown in four replicates of each treatment of the mutagens along with the control seeds in earthen pots filled with soil manure and kept in the net house of the Department of Botany, Aligarh Muslim University during rabi season of 2017–2018.

The experiments were set up to estimate the effect of caffeine and EMS on seed germination, inhibition, plant survival, pollen fertility (pollens were collected from randomly selected young flowers and treated with 2% aceto-carmine solution, and observed under microscope), and relative reduction in pollen fertility in M_1 generation. The followings are calculated using formulae:

$$\text{Germination}\,(\%) = \frac{\textit{Number of seed germinated}}{\textit{Number of seed sown}} \times 100$$

$$\text{Inhibition}\,(\%) = \frac{\textit{Control} - \textit{Treated}}{\textit{Control}} \times 100$$

$$\text{Survival}\,(\%) = \frac{\textit{Number of plant at maturity}}{\textit{Number of seed germinated}} \times 100$$

$$\text{Pollen fertility}\,(\%) = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Permanent slides of meiotic studies were made using the method followed by Shahwar et al. (2018). Microphotographs of freshly prepared slides were taken using X30 Olympus research photomicroscope.

Statistical analysis was done using SPSS 20.0 for Windows (SPSS, Chicago, IL, USA). One way analysis of variance (ANOVA) was calculated through DMRT.

Results

Biological parameters in M_1 generation

The maximum seed germination, plant survival, and pollen fertility gradually decreased in the treated population with increasing concentrations of caffeine and EMS (Fig. 1A, B respectively). The inhibition in seed germination and relative reduction in pollen fertility were found to be increased according to the increasing concentrations of both the mutagens. The seed inhibition was recorded to be minimum in 0.10% treatment of caffeine and EMS and maximum in 1.0% treatment of caffeine and EMS (Fig. 1C). The relative reduction in pollen fertility also showed similar type of effects having minimum and maximum relative reduction in 0.10% and 1.0% of caffeine and EMS respectively (Fig. 1D).

Quantitative traits in M_1 generation

The effect of mutagens on quantitative traits of treated plants is presented in Fig. 2A–F.

The plant height, number of branches/plant, number of pods/plant, number of seeds/pod, 100-seed weight (gm) and total yield/plant (gm) decreased in higher concentrations as compared to control. The plant height showed significant increase in lower doses of caffeine and EMS, whereas a significant decrease was recorded in higher dose of EMS (Fig. 2A). Both the mutagen showed similar type of response in producing total number of branches per plant. Their effect significantly increased the number of branches in 0.1% concentration and decreased in 0.75 and 1.0% concentrations over the control (Fig. 2B).

The average number of pods per plant was 54.14 in control population, and it increased significantly in 0.10–0.75% caffeine and 0.1% EMS while a significant decrease was observed in 1.0% caffeine and 0.75% and 1.0% EMS (Fig. 2C). Average number of seeds per pods increased significantly in lower concentration of caffeine (Fig. 2D).

As far as, average weight of 100 seeds (gm) was concerned, it increased significantly in 0.10–0.75% of caffeine and 0.10% and 0.25% of EMS and significantly decreased in 1.0% of EMS (Fig. 2E). It was recorded that the yield per plant (gm) increased over the control in lower concentration of caffeine (Fig. 2F).

Morphology of agronomic traits

Various morphological variants such as plant height and growth habit was observed when plants were fully matured and leaf variations were observed after 4-5 days of the seed germination. The control plants showed normal leaves (Fig. 3A). The treated plants showed different type of vegetative and cotyledonary variations such as three long and

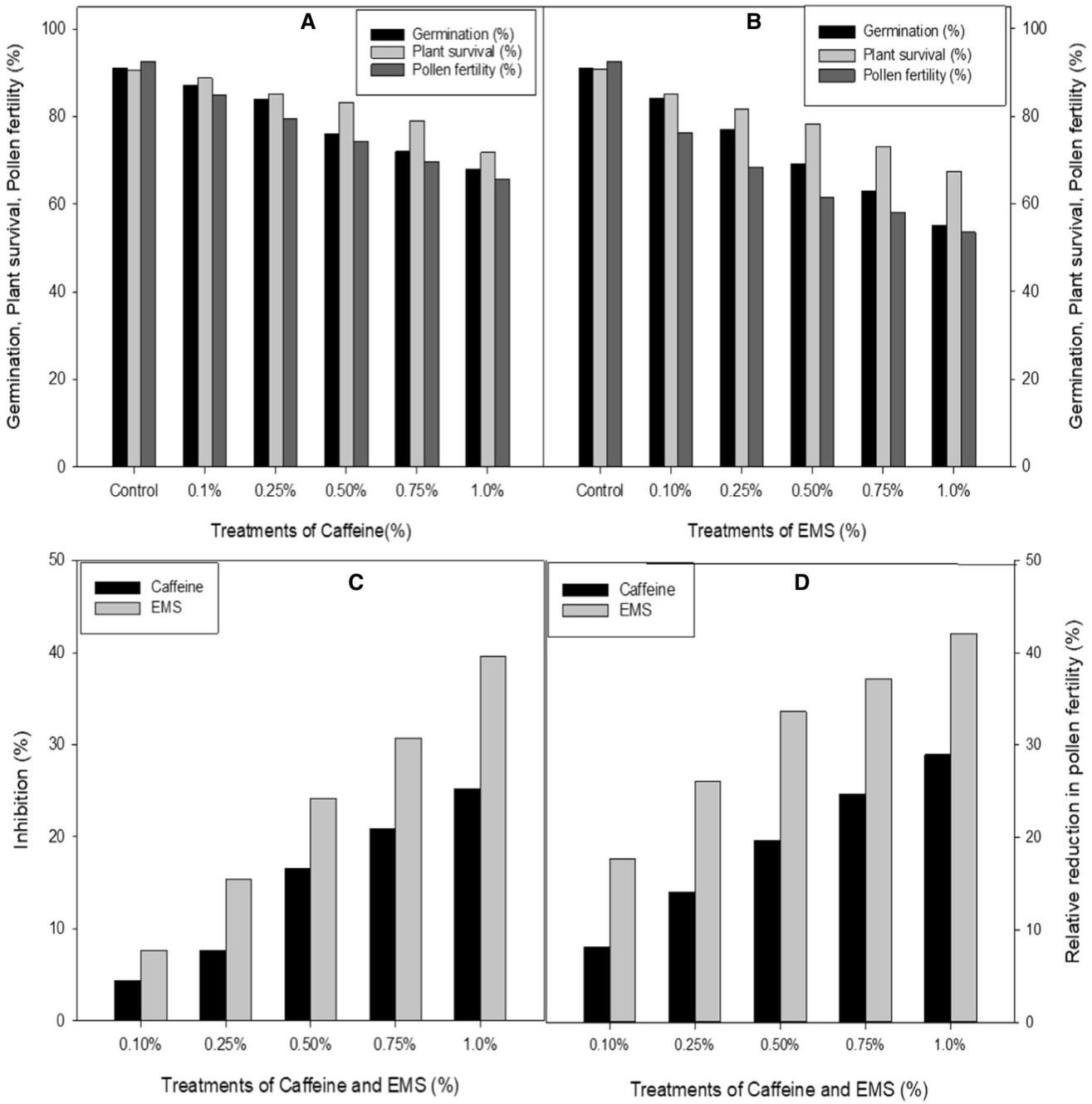

Fig. 1 Illustrates effect of caffeine and EMS on germination, survival, and pollen fertility, inhibition and relative reduction in pollen fertility in lentil L-4076 (M_1 generation)

elongated vegetative leaves (Fig. 3B), two long unequal leaves with one large and broad and other with small and wavy margin (Fig. 3C), leaf with notch at the apex, thick and smooth in surface (Fig. 3D), very small curved leaf (Fig. 3E), one leaf with thick oblong and wavy margin and other small with notch (Fig. 3F), broad heart shaped leaf (Fig. 3G), leaves with chlorophyll mutation (Fig. 3H), leaves having dark green in colour, thick, and with wavy margin (Fig. 3I).

The control population showed normal plant height and yield (Fig. 4A). In mutagenized population, tall and bushy variants were isolated exhibiting increased number of branches with increased size of pods and yield of plants (Fig. 4B). Tall variant were observed in lower concentration of mutagens and showed increased number of branches and pods (Fig. 4C). Dwarf variant were selected in higher

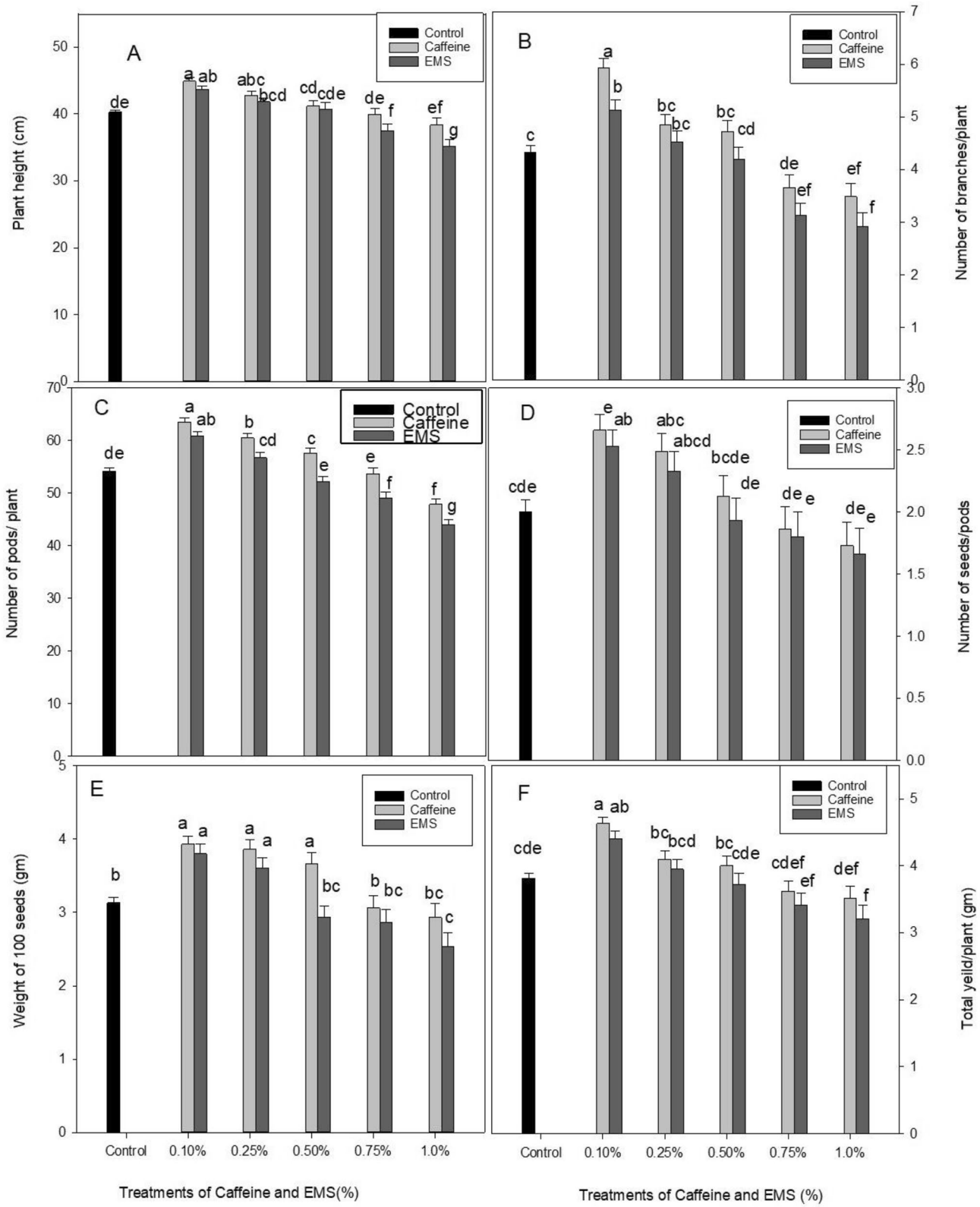

Fig. 2 Illustrates effect of caffeine and EMS on Plant height, yield and its related characters in lentil (M_1 generation). Data means represented by bars followed by the same letter are not significantly different at $P < 5\%$ level of significance, based on the Duncan Multiple Range Test

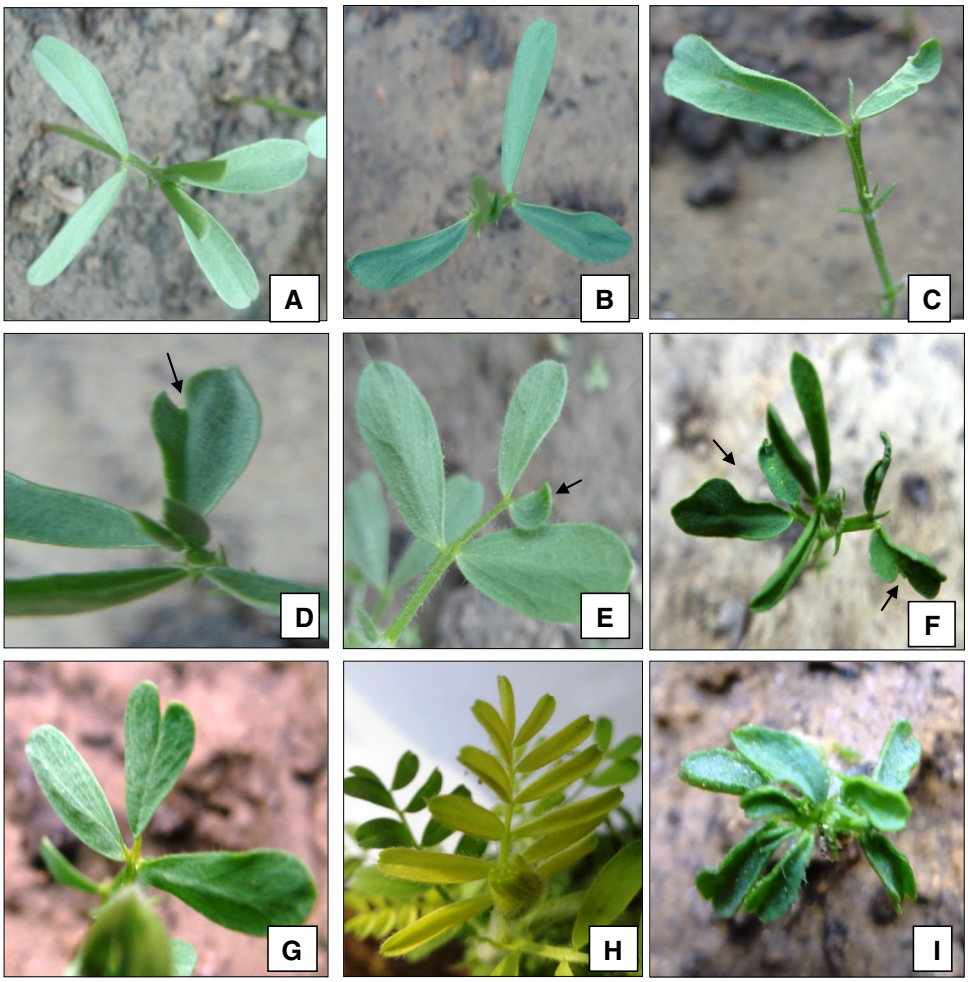

Fig. 3 Different leaves mutant: Four leaves (control) (**A**), Three long and elongated leaves (**B**), Two long uneqal leaves, one large and broad and other small with wavy margin (**C**), leaf showing notch at the apex, thick and smooth in surface (**D**), very small curved leave (**E**), vegetative leaves one was thick oblong with wavy margin and other small and showing notch (**F**), Broad heart shaped leaves (**H**) leaves with chlorophyll mutation (**G**), leaves showing dark green in colour, thick, with wavy margin (**I**)

dose of EMS and showed late maturity with no fruit setting (Fig. 4D).

Cytological studies

In present investigation, meiotic studies of control as well as treated plants were performed to analyse the chromosomal variations produced by the mutagenic treatment. Normal meiosis was observed in control plants such as diakinesis (showed 7 ring bivalents PMCs), anaphase I and telophase II (Fig. 5A–C), whereas, different types of chromosomal abnormalities in treated plants like laggard at telophase I (Fig. 5D), desynchronization at metaphase II (Fig. 5E), disturb polarity at anaphase II (Fig. 5F), laggard at telophase II (Fig. 5G) and disturbed polarity with one micronuclei at telophase II (Fig. 5H) were observed. The frequencies of these aberrations were increased with the increasing concentration of mutagens and highest frequency was recorded in EMS (Table 1 and Fig. 6).

Discussion

Mutation breeding technique has emerged as the best method to enhance the genotypic variation of plant species within a short span of time and has played a pivotal role in the development of many crop varieties. In present study, the genotype of lentil was subjected with chemical mutagens caffeine and EMS to evaluate their genotypic and morphological variability induced by them.

Biological parameters in M_1 generation

During the present investigation, germination, plant survival and pollen fertility decreased as the concentration of mutagens increased. Germination is a process in which after a period of dormancy seeds initiates its growth. Seed germination is metabolic pathways apparent for visible growth and an important parameter to study the effect of mutagenic treatments on plants (Shah et al. 2008). Reduction in germination percentage may be due to effects of mutagen on the activity of gibberellic acid (GA_3) and the

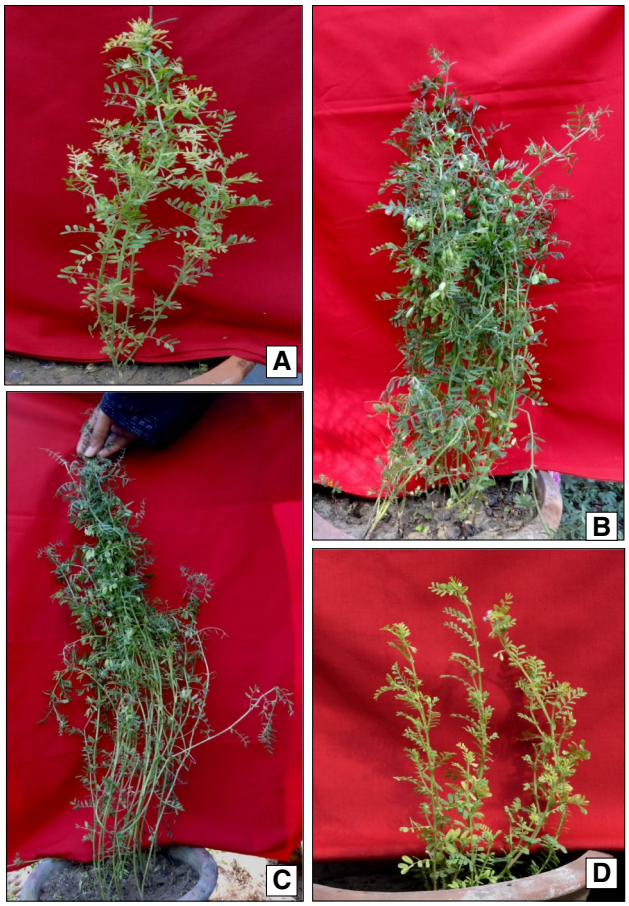

Fig. 4 Control plant with normal height and yield (**A**), Tall and bushy variant with large pod and high yield (**B**), Tall variant with increase number of branches and pods (**C**), Dwarf variant with no fruit setting, late maturity (**D**). (All photographs were taken when plants were fully mature, approximately after 4 months of seed sowing)

seed cell system or it may also be due to genic or chromosomal aberrations (Natarajan and Shivasankar, 1965; Sato and Gaul, 1967). Similar decrease in seed germination have also been reported in *Vigna mungo* (Goyal and Khan 2010) in *Capsicum annuum* (Aslam et al. 2017), in *Vicia faba* (Shahwar et al. 2016). The plant survival was found to decrease in treated plants with increasing concentration of mutagens. Similar decrease in plant survival have also been reported in *Cajanus cajan* by Potdukhe and Narkhede (2002), in *Vigna radiata* by Auti (2005), in *Glycine max* by Kavithamani et al. (2008), in *Vigna unguiculata* by Dhanavel et al. (2008) and in *Lens cilinaris* by Shahwar et al. (2020) as observed in this present investigation. Physiological imbalance or chromosomal aberrations due to chemical mutagens may be attributed to the decrease in seedling survival. Pollen fertility was observed to be inversely related with the dose of mutagen and it decreases with increasing concentration of mutagens. Similar findings were reported by Dixit et al. (2013) in *Nigella sativa* due to the effect of EMS and sodium azide (SA). Chemical mutagens produce different type of chromosomal abnormalities which may lead to production of sterile pollen grains resulting in decrease of pollen fertility (Muthusamy and Jayabalan 2002 in *Gossypium hirsutum* and Kumar and Rai 2007 in *Glycine max*).

Quantitative traits in M_1 generation

In present study, plant height found to be increased over the control in lower concentration and reduced in higher concentration of mutagens, this reduction is may be due to chromosomal aberrations produced by the mutagens (Kumar and Tripathy 2008). Reduction in plant height may also be attributed to physiological disorders such as auxin reduction caused by mutagens (Krishna et al., 1984) or due to the metabolic disruption of plant. Similar result has been reported by Talebi and Talebi (2012) in *Oryza sativa* and Ambavane et al. (2015) in *Eleusine coracana*. Number of branches per plant increased in lower concentrations of mutagen. Similar increase in number of primary branches were also reported by Charumathi et al. (1992) in *Vigna mungo*, Waghmare and Mehra (2000) in *Lathyrus sativus* and Khan et al. (2005) in *Cicer arietinum*.

The ultimate motive of plant breeder is to increase the yield along with other beneficial characters. In present study, at lower doses of mutagen yield and its related characters showed stimulatory effects while at higher doses they exhibited inhibitory effect. Similar results were observed by Ali and Shaikh (2007) in *Hordeum vulgare,* Sinha and Lal, (2007) in *Lens culinaris*, Khursheed et al. (2009) in *Helianthus annuus*, and Meshram et al. (2013) in *Vigna mungo*. The significant increase in yield and other traits may attribute due to stability in gene mutation in subsequent generations (Panigrahi et al. 2015).

Morphology of agronomic traits

Beneficial mutations in different agronomic traits play a crucial role in mutation breeding programmes. In the present investigation, tall variants were recorded at lower concentration in M_1 generation. It was also observed by Shahwar et al. (2017) in *Vicia faba*, and by Khursheed and Khan (2014) in lentil. Dwarf variant was also recorded in M_1 generation. They showed small internode that was probably due to disturbance in cell division. Many researchers also recorded about dwarf plants like Talukdar and Biswas (2007), Arulbalachandran and Mullainathan (2009), and Wani et al. (2008). In present investigation, tall and bushy variant with high yield was isolated similar to lentil by Shahwar et al. (2017). Morphological variant of leaves such as size (small and large) and shape (narrow and broad) of leaflets were

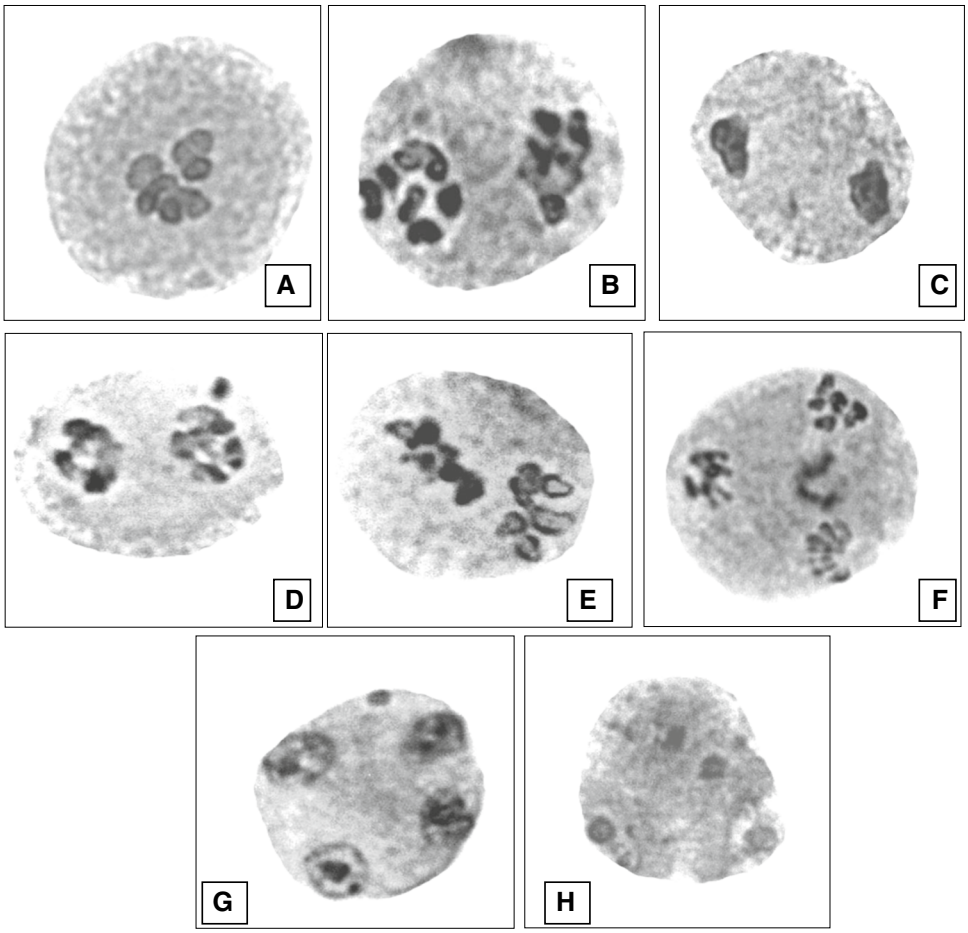

Fig. 5 Chromosomal abnormalities- diakinesis (control) (**A**), Anaphase I (control) (**B**), telophase I (control) (**C**), laggard at telophase I (**D**), desychronization at metaphase II (**E**), Disturb polarity at anaphase II (**F**), one laggard at telophase II (**G**), disturbed polarity with one micronuclei at telophase II (**H**)

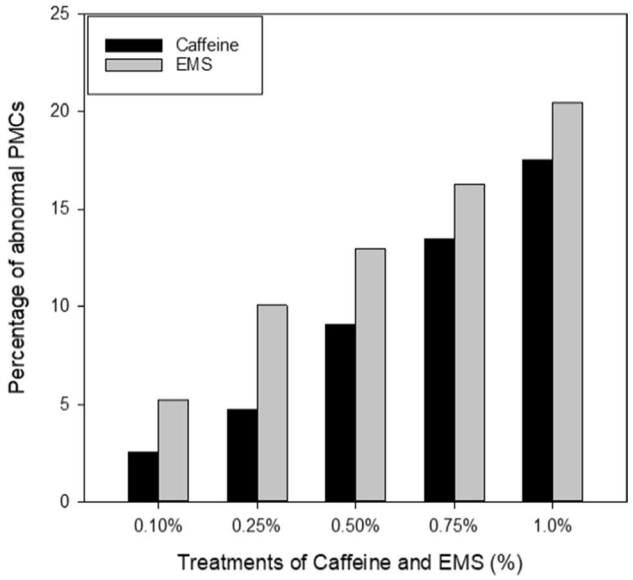

Fig. 6 Effect of different treatments of caffeine and EMS on percentage of total chromosomal aberrations in lentil

observed. Such results also reported in lentil by Shahwar et al. (2019) and Shah et al. (2006) in Chickpea.

Cytological studies

In present study, different types of chromosomal aberrations were observed like univalent, multivalents, stickiness, laggard, desynchronization and disturb polarity. Cytological studies approaches for selection of desirable traits by specific responses of different genotypes to a specific mutagen (Avijeet et al. 2011). Unequal division of chromosome or reduction of complete set of chromosome is due to movement of univalent or bivalent toward one pole Khan et al. (2009). Mutagens may disturb the nucleoprotein backbone by altering proteins induced by their toxic effects which may leads to precocious separation of chromosomes (Kumar and Rai 2007). Laggards at anaphase I/II occurred probably due to the delayed terminalization of chiasma or due to stickiness of chromosomal ends (Minija et al. 1999). Similar findings have also been reported by Alka et al. (2012) in *Linum usitatissimum*, and Choudhary et al. (2012) in *Trigonella foenum-graecum*. Disturbed polarity may be attributed to disturbances in spindle fibre which may lead to multinucleate

Table 1 Frequency of meiotic aberrations induced by caffeine and EMS in *Lens culinaris* Medik. (M_1 generation)

Conc.of mutagens (%)	Total No. of PMCs Obser	Prophase-I (Diakinesis			Metaphase-I/II						Anaphase-I/II			
		Univalents	Multivalents	% of Abn. PMCs	Univalents	Multivalents	Precocious Mov	Stray chromosome	Stickiness	% of Abn. PMCs	Laggards	Bridges	Unequal Sep	% of Abn. PMCs
Control	275	–	–	–	–	–	–	–	–	–	–	–	–	–
Caffeine (%)														
0.10	236	–	–	–	–	1	–	–	1	0.84	1	–	–	0.42
0.25	252	–	1	0.39	–	1	–	1	2	1.58	1	–	1	0.79
0.50	263	1	2	1.14	1	2	1	1	2	2.66	2	1	1	1.52
0.75	275	2	2	1.45	2	2	2	2	3	4.00	2	2	2	2.18
1.0	285	3	3	2.10	3	3	2	3	3	4.91	3	2	3	2.80
EMS (%)														
0.10	247	–	1	0.40	–	1	–	1	1	1.21	1	–	1	0.80
0.25	258	1	2	1.16	1	2	1	2	2	3.10	2	1	1	1.55
0.50	262	1	2	1.14	1	2	2	3	2	3.81	2	1	2	1.90
0.75	270	2	3	1.85	2	3	2	3	3	4.81	2	2	2	2.22
1.0	278	3	3	2.15	3	4	4	4	5	7.19	3	2	3	2.87

Conc.of mutagens (%)	Telophase-I/II								Total No. of Abn. PMCs Obser	Total % of Abn. PMCs Obser
	Laggards	Bridges	Unequal Sep	Micro nucleate	Multi nucleate	Disturbed polarity	Cytomixis	% of Abn. PMCs		
Control	–	–	–	–	–	–	–	–	–	–
Caffeine (%)										
0.10	1	–	1	–	–	1	–	1.27	6	2.53
0.25	1	–	1	1	–	1	1	1.98	12	4.74
0.50	2	1	2	1	1	2	1	3.80	24	9.12
0.75	3	2	2	2	2	3	2	5.81	37	13.44
1.0	4	2	3	3	3	4	3	7.71	50	17.52
EMS (%)										
0.10	1	–	1	1	1	2	1	2.83	13	5.24
0.25	2	1	1	2	2	2	1	4.26	26	10.07
0.50	3	1	2	2	3	3	2	6.10	34	12.95
0.75	3	2	2	3	4	4	2	7.40	44	16.28
1.0	4	2	3	3	4	4	3	8.27	57	20.48

cells. Similar abnormalities were also reported by Sharma et al. (2009), Bhat et al. (2012), Khan et al. (2019) and Shahwar et al. (2020).

Conclusion

From the present investigation it can be concluded that the mutagens caffeine and EMS generates variety of variants in lentil. Most of the useful and significant mutations were observed at lower concentration of caffeine and EMS. The moderate dose of mutagens accelerates the rate of desirable high yielding variants which proved to be economical. These selected variants in future generations will definitely contribute towards the further improvement of genotype of lentil and may be used as valuable breeding stocks for lentil breeding.

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