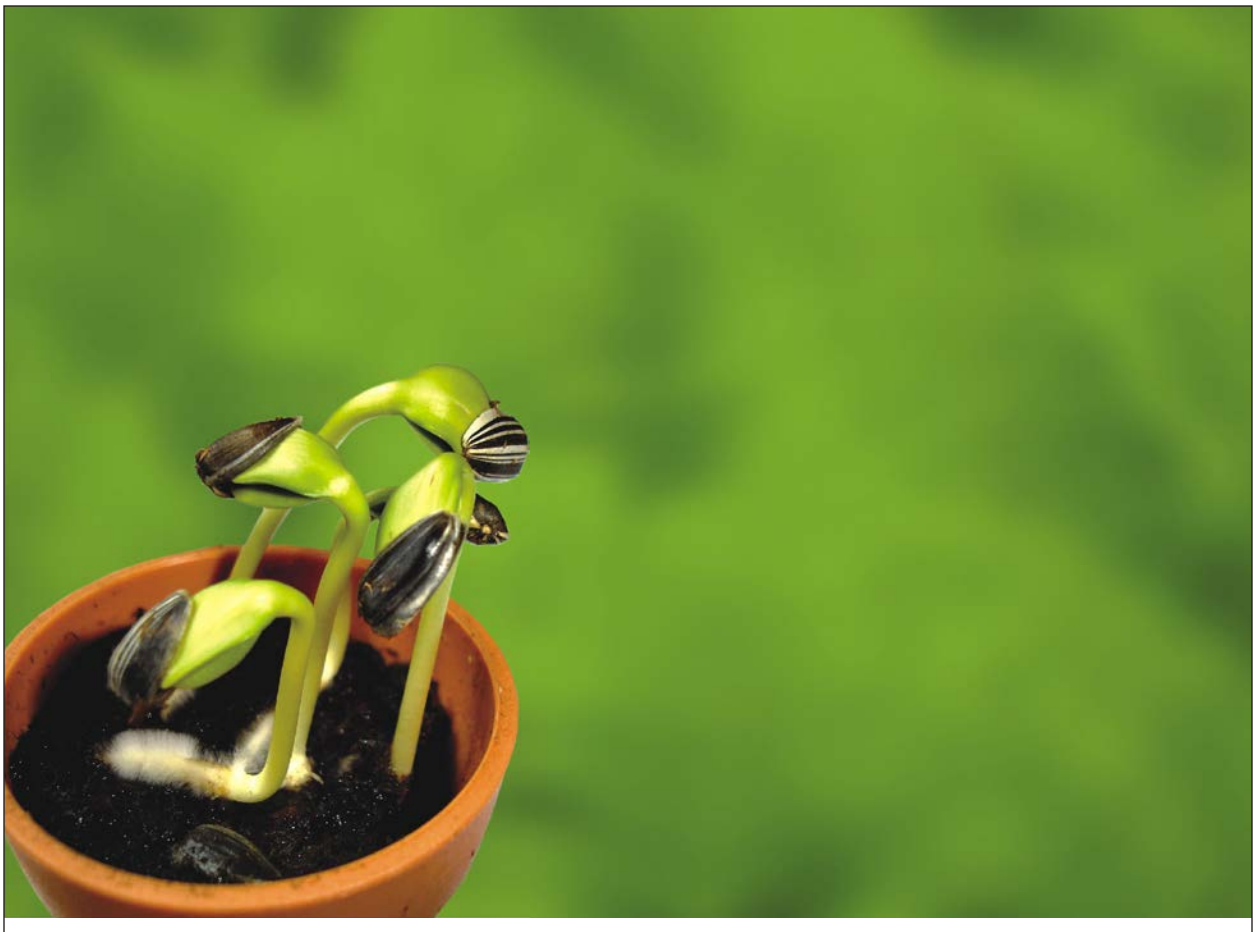

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Research Article
Evaluation of High Yielding Mutant of Lentil Developed Through Caffeine of an Exotic Germplasm

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Abstract

Background and Objectives: Genetic variation is indispensable to any plant improvement programme, therefore; this study was primarily based on this aspect of inducing desirable genetic variation for enhancement of the available lentil genetic diversity. The objective of study was to find out the response of caffeine on lentil with a view to distinguished the effect of chemical mutagen on quantitative and qualitative traits in M_2 generation. **Materials and Methods:** In the present experiment, potent chemical mutagen caffeine was used to raise a viable and beneficial mutant at M_2 generation level. The seeds (genotype) of lentil were treated with 0.1, 0.25 and 0.50% dose of caffeine prepared in phosphate buffer. **Results:** Different qualitative and quantitative traits of mutant plant were screened and considerable variations in phenotypic characters were observed. Morphology of plant which is high yielding than its wild type is altered by wide range of macro mutations are called high yielding mutant at 0.25% dose of caffeine. Cytological studies were also done in control as well as mutant plant. Different cytological anomalies such as disturb metaphase with two stray chromosome and stickiness were found. Laggards disturb metaphase II non synchronization and disturbed polarity was also observed in high yielding mutant plant. **Conclusion:** The optimal dose of caffeine showed accelerating rate of desirable high-yielding mutant had proved to be economical. The segregate of the selected mutant in future generations will definitely contribute to the improvement of lentil genotype and this mutant may be used as valuable breeding stocks for lentil breeding. So by using chemical mutagenesis breeders develop induced mutants in different plants.

Key words: Chemical mutagenesis, high yielding mutant, genetic variation, caffeine, cytological aberration, chromosomal lesion

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

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

INTRODUCTION

Lentil (*Lens culinaris* medik) is diploid 2n = 14, an early domesticated plant species, as old as those of einkorn, emmer, barley and pea[1]. It is an annual herb, belongs to legume family named as fabaceae. This habit grows as a slender bush or twine vine with 0.5 m height, also bears alternate pinnate leaflets, terminal solitary or in clusters white to bluish flower. The fruit is small flattened, pods containing one or two lens shaped seeds. Among pulse crop, lentil is of special interest with 26% content of grain protein. In spite of this, seed is valuable source of carbohydrates, minerals vitamins and bioactive peptide, health polyphenol in combination with Glycemic Index (GI). Lentil is rich sources of dietary fibers, resistant starches, prebiotic compound, phytochemicals, phenolic acids and antioxidant consumed by human as food. While the straw serve as high value animal feed[2]. Genetic variation is the currency of any crop improvement experiment and it is the long history of conventional legume breeding that causes the genetic bottlenecks which affects yield and quality. Mutation breeding is the only feasible and sustainable technique to broaden their narrowing genetic bases to create a gene pool of numerous desirable traits of economic importance. Chemical mutagenesis is coherent tool used in mutation breeding program for creating new alleles[3] and is economically sound to be performed on small and large scale[4].

Development of mutants using chemical mutagenesis has broadened the boundaries of improvement of vegetative and reproductive traits, which synergistically can enhance the yield and quality. Mutation breeding programmes have become an inherent tool for the development of determinate plant type with improved yield, seed size, grain quality, non shattering plants[5], high yielding and disease resistant[6], early maturing[7] and others by using chemical mutagens. In several cases mutagens cause chromosomal instability which lead to apoptosis but may not always be harmful. Lower and moderate doses of mutagens stimulate DNA repairing. Caffeine (Fig. 1) possesses safe threshold at its optimal concentration among various mutagens. It possesses significant ability to improve qualitative and quantitative traits in plants[8] and reduces carcinogenic risk due to dietary and environmental effects[9].

Pulses are mainly associated with human beings and form an important constituent of food. It is cultivated for its seeds and mostly eaten as food supplement. Development of lentil mutant (high yielding) line which could serve the increasing population demands need to be established for its high protein content. The current approach of inducing phenotypic diversity and generation of morphological mutants of lentil using chemical mutagenesis may help in modifying the different traits for using in cross-breeding programmes during the future crop improvement programmes. So, in this aspect high yield mutant can serve better over its wild type. Thus, in the present work, the strategy was to achieve potential mutations using optimal doses of caffeine via chemical mutagenesis to serve mankind through these important pulses.

MATERIALS AND METHODS

Chemical solutions used:

- Stock solution of caffeine (1% v/v respectively) prepared in phosphate buffer
- Buffer tablets
- Carnoy's fixative (absolute alcohol: chloroform: acetic acid 6:3:1 v/v ratio)
- Absolute alcohol (70%)
- Propionocarmine stain
- Canada balsam

Mutagenic treatment: The seeds of *Lens culinaris* variety L-4076 were obtained from Indian Agriculture Research Institute (IARI), New Delhi, India. Fresh aqueous stock solutions of caffeine (1% v/v respectively) manufactured by Sissco Research Laboratories Pvt. Ltd, Mumbai, India, were prepared in phosphate buffer at pH 7.0. The pH of the solution was maintained using buffer tablets (MERCK manufactures, Mumbai, India). The fresh, healthy and uniform seeds were presoaked in distilled water for 12 h and then treated with 3 different concentrations (0.1, 0.25 and 0.50%) of stock solution of caffeine for 8 h with intermittent shaking. After treatment, the seeds were thoroughly washed in running tap water for 30 min to remove the excess of mutagen. Thoroughly washed 50 seeds were sown in four replicates of each treatment of the mutagen as well untreated seed (control) in earthen pots filled with soil manure and kept in the Net House of the Department of Botany, Aligarh Muslim University during the Rabi season of the year 2015-2017 to raise M_1 and M_2 generation.

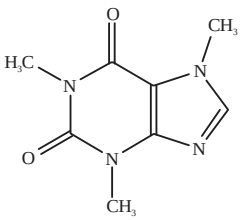

Fig. 1: Chemical structure of caffeine ($C_2H_{10}N_4O_2$)

Morphological screening: Morphological screening was done time to time after the seed germination and the preliminary screening for phenotypic characteristics was done regularly to monitor any mutated character in the plant morphology. On maturity, M_1 seeds were collected and M_2 generation was raised from all harvested seeds during next year of November and different qualitative and quantitative traits were showing maximum deviation in characters.

Cytological screening: For cytological examination, young flower buds of appropriate size from control as well as mutant plants were selected and fixed separately in Carnoy's fixative (absolute alcohol: chloroform: acetic acid 6:3:1 v/v ratio) for 24 h and then stored in 70% alcohol. Anthers were squashed in 1.0% propionocarmine for staining; slides were made permanent through an NBA-GAA series, mounted in Canada balsam and dried at 45°C. Different meiotic stages were recorded through photographs using a high resolution (Dsx 100 Olympus) Microscope made in India.

RESULTS

Interpreting the effect of different concentrations of caffeine on various morphological characters, qualitative and quantitative traits, a "High yielding mutant" was recovered in M_2 generations at 0.25% dose of caffeine. The mutant is high yielding than control.

Qualitative and quantitative traits: High yielding mutant took more number of days for germination, flower initiation, 50% flowering and maturity than control which is comparable with all these quantitative traits (Table 1). An account of qualitative traits the mutant was also found superior over control showing vigorous growth and high yield (Fig. 2a), bifurcate and bilobed leaves (Fig. 2b) flower bearing white with light blue vein, three flowers per peduncle as compared to two flowers per peduncle in control (Fig. 2c, Table 1). The pod and seeds size was also larger than the control (Fig. 2d, e, Table 1). However, the seeds were light brown in both cases in control as well as in high yielding mutant (Fig. 2d, Table 1).

Morphological parameters: Different morphological parameters were constructed between control and mutant plant (Table 2). The mutant is taller, increasing the height (cm) as compare to control (Table 2). Number of branches per plant was also increased than control plant. In mutant plant yield related characters were also enhanced as compare to control (Table 2). High yielding mutant was bearing higher number of pod per plant and larger pod length than control plant. Increased number of seeds per pod and yield/plant was found in mutant plant over in control plant (Table 2).

Meiotic studies: On the examination of microsporogenesis, meiosis was observed to be normal in control plant showing diakinesis, metaphase I, telophase I, telophase II (Fig. 3a-d), whereas in high yielding mutant several chromosomal lesions were seen during cell cycle. The meiotic abnormalities were recorded in mutant plant such as disturbed metaphase with two stray chromosome (Fig. 3e), sticky metaphase I (Fig. 3f), laggard at telophase I (Fig. 3g), disturbed metaphase II non synchronization (Fig. 3h), I-Disturbed polarity at anaphase II (Fig. 3i).

DISCUSSION

For induction of mutant in plants, chemical and physical mutagens have been used since long time. On account of it, mutagenesis has been used to produce mutant by various researchers[10-14]. The present study has demonstrated that it was beneficial for the induction of high yielding mutant on the

Table 1: Salient qualitative and quantitative character to distinguish between control and high yielding mutant

Traits	Control	Mutant
Qualitative traits		
Plant habit	Erect, herb	Erect, herb
Growth	Normal	Vigorous
Flower	2 flowers per peduncle	3 flowers per peduncle
Flower color	White with blue vein	White with light blue vein
Pod	Normal	Large
Seed size (cm)	Normal	Big
Seed color	Brown	Brown
Quantitative traits		
Number of days to germinate	2-3	4-5
Number of days to flower initiation	86	97
Number of days to 50% flowering	105	122
Number of days to maturity	135	150

Fig. 2(a-e): Morphological variation between control and high yielding mutant of *Lens culinaris*, (a) 1: Control plant, 2: High yielding mutant plant, (b) 1: Vegetative leaf control, 2: Vegetative leaf mutant, (c) 1: Flower control plant, 2: Flower mutant, (d) 1: Pod control, 2: Pod mutant and (e) 1: Seeds control, 2: Seeds mutant

Table 2: Morphological parameters of control and high yielding mutant

Parameters	Control	Mutant
Plant height (cm)	39.00	46.00
Number of branches per plant	3.00	5.00
Number of pods per plant	49.00	58.00
Pod length (cm)	1.00	1.30
Number of seeds per pod	2.00	3.00
Yield per plant (g)	2.63	3.17

screening of M_2 generation which indicates the potential impact of caffeine on *Lens culinaris*, which might be as a result of pleiotropic effects of mutated genes or chromosomal aberrations or gene mutation. The quantitative characters are improved by the result of pleiotropic effects of mutated genes or chromosomal aberrations. In recent years, mutation breeding is used to increase the variability in quantitative characters[15,16]. Several works have been done on lentil (*Lens culinaris*) for evaluation of useful mutants as a result of chemical mutagenesis. Khursheed *et al.*[17] suggested that lower doses of caffeine exhibited stimulatory effect on qualitative and quantitative traits of sunflower. In present work, caffeine has shown its ameliorative action for modulating useful pulse crop with meiotic stability. Mutant evaluated due to stimulatory effect of caffeine exhibit increased height with higher number of branches. This is because of loss of apical dominance which results in lateral distribution of growth hormone and hence increased branching. Gnanamurthy and Dhanavel[18] reported that mutant bearing increased height

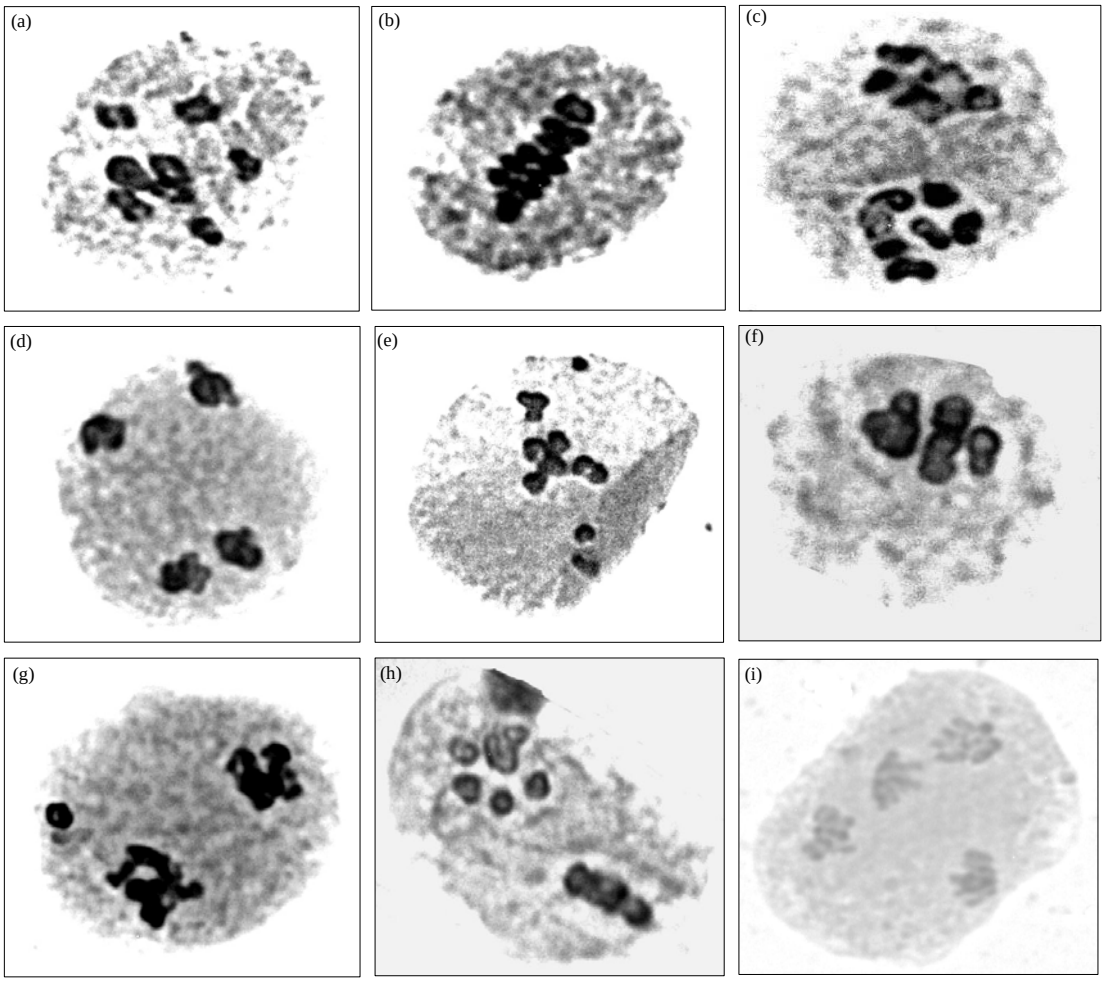

Fig. 3(a-i): PMC showing (a) Diakinesis, (b) Metaphase I, (c) Telophase I, (d) Telophase II (control), (e) Disturbed metaphase with two stray chromosome, (f) Sticky metaphase I, (g) Laggard at telophase I, (h) Disturbed metaphase II non synchronization and (i) Disturbed polarity at anaphase II (mutant)

and branches by treatment of ethyl methane sulfonate in cowpea. In this case isolated mutant could be highly useful because mutant produced higher yield than control. Total yield per plant is directly related with number of pods per plant because whenever number of pod per plant increased, total number of seeds increased and hence yield also increased. The variation in yield contributing characters due to different doses of mutagens as observed in *L. culinaris*[19-22], *Triticum durum*[23], *Vigna radiate*[24]. In mutation breeding, yield is very important parameter because the plant breeders want to improve the yield along with other characters. These characters were higher at lower concentration but it shows reduction pattern at higher doses. It was also suggested that enhancement of yield at moderate level and variations in quantitative parameters may shows stable gene mutation in the next generation by Panigrah *et al.*[25] while Thilagavathi and Mullainathan[26] concluded that the decrease in quantitative traits have been attributed to the physiological disturbance or chromosomal damage caused to the cells of the plant by mutagens. Moreover, the mutant has three flowers per peduncle instead of two flowers (in control plant) which is index of increased number of seeds, increased size of seed and better yield. The similar character was noticed by Amin *et al.*[10] in lentil by mutagenic effect of MMS with 2% DMSO. Specific mutagens exhibit specific response of different genotype to provide the significant evidence for selection of desirable traits in cytological investigation[27]. In the present experiment, the screened mutant has shown chromosomal lesion such as disturbed metaphase with two stray chromosomes, sticky metaphase I, laggard at telophase I, disturb metaphase II non

synchronization, disturbed polarity at anaphase II. Stray` chromosomes at metaphase-I seem to be caused by spindle dysfunction and clumping of chromosomes[28]. Disturbed polarity at anaphase and telophase stages could be due to spindle disturbance. Disturbed polarity was also reported in maize[29], *Trigonella*[30] and *Vicia faba*[31]. Stickiness was found clump into one, two or many groups at metaphase causing difficulty in the normal disjunction of chromosomes. These results are in agreement with those in *Vicia faba*[31], *Capsicum annuam*[32,33] and *Linum usitatissimum*[34]. Gaulden[35] attributed chemically induced stickiness to the direct action of the mutagen on histone proteins leading to improper folding of DNA. According to Tarar and Dnyansagar[36] unsynchronized bivalents or laggards might be due to discrepancies in spindle formation. Presence of laggards may be attributed to the inability of multivalents to separate properly[37]. Laggard may be arising by breakage or faulty spindle resulting into imbalanced daughter nuclei and micronuclei[38].

The findings of this mutation breeding study provide an extensive research of the caffeine mutagenesis and effect of this in lentil. The investigation postulates that caffeine as a useful chemical mutagen to create the required supplementary variation can be recommended to lentil mutation breeding program. Moderate concentrations of caffeine resulted the biological damages while increasing the frequency of desirable mutants and ultimately yield in the treated population of lentil.

CONCLUSION

It is concluded that caffeine generates a variety of mutant in *Lens culinaris*. It is noticed that these changes are differentially sensitive to caffeine and the appearance of new mutant would be very helpful in maintaining the genetic purity of plant variety. Therefore, this mutant should be isolated after chemical mutagenesis and desired character needs to be selected from it. The cytological analysis of these mutants showed that these changes were induced due to changes in chromosomal number, structure, base substitution and deletion.

SIGNIFICANCE STATEMENTS

This study finds out the high yielding mutant, through induced mutagenesis and breeding program, having practical utility to plant breeders as it is high yielding with better qualitative traits coupled with increased height (cm), number of branches and yield related traits. This study will help the researcher to uncover the critical areas of mutation breeding program for crop improvement to develop viable mutants plant that could be beneficial for human being, that many researchers were not able to explore. Thus by using micro and macro mutation, researchers need to broaden their narrowing genetic bases to boost the productivity and create a gene pool of numerous desirable traits of economically important crops.

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