Asian Journal of Plant Sciences

ISSN 1682-3974

OPEN ACCESS

Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2017.221.226

Research Article
Induced Chlorophyll Mutations, Comparative Mutagenic Effectiveness and Efficiency of Chemical Mutagens in Lentils (*Lens culinaris* Medik)

Mohammad Rafiq Wani

Department of Botany, Government Degree College (Boys), 192102 Anantnag, Jammu and Kashmir, India

Abstract
Background and Objective: Chlorophyll mutants are used as markers in genetic, physiological and biochemical investigations. The selection of effective and efficient mutagen(s) is very essential to recover high frequency and spectrum of desirable mutations. Present investigation was undertaken to understand the response of lentil variety Pant L-406 to various chemical mutagens for identifying such mutagenic-treatment causing maximum chlorophyll mutations which could eventually be exploited for inducing viable and economically useful mutations in subsequent generations. **Materials and Methods:** Seeds of lentil were treated with chemical mutagens namely methylmethane sulphonate (MMS), hydrazine hydrate (HZ) and sodium azide (SA) for 6 h. Three replications of 100 seeds per treatment were sown in complete randomized block design (CRBD) to raise M_1 generation. The M_1 plants were harvested separately and seeds sown in next season in plant progeny rows to raise M_2 generation. Chlorophyll mutations were observed in M_2, when seedlings were 8-15 days old. The frequency of chlorophyll mutations, mutagenic effectiveness and efficiency were determined as per the standard formulae. **Results:** Different types of chlorophyll mutants viz., albina, xantha, chlorina, maculata and virescent were observed in the field when seedlings were 8-15 days old. All these chlorophyll deficient mutants were lethal except maculata and virescent which produced few seeds at maturity. The MMS treatments induced the highest frequency of chlorophyll mutations followed by HZ and SA. The frequency of chlorophyll mutations was dose dependent and increased with increasing concentrations of the mutagens. 'Chlorina' followed by 'xantha' outnumbered the other types of chlorophyll mutants in all the mutagens. Higher effectiveness was recorded with HZ treatments, whereas SA was found to be the most efficient mutagen compared to HZ and MMS. **Conclusion:** Chlorophyll mutations, although do not have much economic importance due to their lethal nature, such a study could however, be useful in identifying the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutants in subsequent generations.

Key words: Chemical mutagens, chlorophyll spectrum, effectiveness and efficiency, lentil, sodium azide

Received: July 11, 2017 Accepted: August 31, 2017 Published: September 15, 2017

Citation: Mohammad Rafiq Wani, 2017. Induced chlorophyll mutations, comparative mutagenic effectiveness and efficiency of chemical mutagens in lentils (*Lens culinaris* Medik). Asian J. Plant Sci., 16: 221-226.

Corresponding Author: Mohammad Rafiq Wani, Department of Botany, Government Degree College (Boys), 192102 Anantnag, Jammu and Kashmir, India Tel: +919906517207

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 one of the most important legume crops worldwide including India. India is the largest producer of lentil and contributes to about 32% of total lentil production in world. Of late, due to stagnation and decreasing production and rapid increase in population, the net availability of total pulses has come down from 60 g/day/capita in 1951 to 37 g/day/capita now although India ranked 23rd in lentil productivity[1,2]. Besides stubble, which is also rich in many valuable components, the grains are composed of 60% of carbohydrates, 26% protein, 7.5% of iron, 2% of sugars and 0.87% of thiamine (vitamin B_1) which are essential ingredients of both human and animal feeds[3,2].

Mutation breeding has been considered as one of the gizmo of atomic age and a much promising breeding short cut which could be beneficially utilized for tailoring the desired varieties of different crop plants. The major advantage of the use of induced mutations is the possibility to correct one or few negative characters of a cultivar, without changing the major parts of its total genetic set up. Mutation breeding has now become a novel and eco-friendly but useful and in some situations even an indispensable tool which can be employed by the plant breeders[4].

The enhancement of mutation frequency and alteration of mutation spectrum in a predictable manner are the two important goals of mutation research. In past, varied approaches have been tried to achieve these goals[5-7]. Chlorophyll mutations are considered as the most dependable indices for evaluating the genetic effects of mutagenic treatments and are also used as genetic markers in basic and applied research. Several authors have reported the incidence of different types of chlorophyll mutations in M_2 generation following treatments with different mutagenic agents in various crop plants[8-20].

Before the start of any sound breeding programme, knowledge of relative biological effectiveness and efficiency of various mutagens and their selection is essential to recover high frequency of desirable mutations[21,22]. It is not necessary that an effective mutagen shall be an efficient one also[23-25]. Mutagenic effectiveness relates mutagen dose to the mutational events, while mutagenic efficiency gives an idea of genetic damage in relation to the total biological damage caused in M_1 generation[26]. Though both of these are two different properties but the usefulness of any mutagen in plant breeding programme depends on both of them. The response of biological system to mutagens is influenced to a varying degree by numerous biological, environmental and chemical factors. These factors modify the effectiveness and efficiency of different mutagens greatly and same is true for the mutation rate[27-29]. The present investigation was undertaken with the aim to study the effects of MMS, HZ and SA on the frequency and spectrum of chlorophyll mutants and to evaluate the relative effectiveness and efficiency of these chemical mutagens in M_2 generation of lentil variety Pant L-406.

MATERIALS AND METHODS

A field experiment was conducted during the rabi season of 2006-2007 and 2007-2008 at University Agricultural Farm, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Dry and healthy seeds of lentil (*Lens culinaris* Medik) variety Pant L-406, pre-soaked in distilled water for 9 h, were treated with three chemical mutagens viz., methylmethane sulphonate (MMS): A monofunctional alkylating agent manufactured by Sissco Research Laboratories Pvt. Ltd., Mumbai, India, hydrazine hydrate (HZ): A base analogue, manufactured by Qualigens Fine Chemicals, Mumbai, India and sodium azide (SA): A respiratory inhibitor, manufactured by Indian Drugs and Pharmaceuticals Ltd., Hyderabad, India for 6 h. The concentrations used for MMS ranged from 0.1-0.4%, whereas these were 0.01-0.04% for HZ and SA. The solutions of MMS and HZ were prepared in phosphate buffers of pH 7, whereas SA solution was prepared in phosphate buffer adjusted to pH 3. To facilitate uniform absorption, large quantities of the solution of mutagens, approximately three times the volume of seeds were used[26]. Following these treatments, the seeds were thoroughly washed in running tap water to remove the residue mutagens from the seed surface. The treated seeds were directly sown in the field along with untreated control. Three replications of 100 seeds/treatment were sown in complete randomized block design (CRBD) to raise M_1 generation. The M_1 plants were harvested separately and the seeds sown the next season in plant progeny rows to raise M_2 generation. The distance between the seeds in a row and between the rows was kept at 30 and 60 cm, respectively.

Chlorophyll mutations were scored in M_2 generation when seedlings were 8-15 days old. They were identified and classified according to Gustafsson[30]. The frequency of chlorophyll mutations was calculated as per Konzak *et al.*[26]:

$$\text{Mutation frequency (\%)} = \frac{\text{No. of mutant seedlings}}{\text{Total No. of } M_2 \text{ seedlings}} \times 100$$

Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of a mutagen (time×concentration), while mutagenic efficiency represents the proportion of mutations in relation to biological damage. Formulae suggested by Konzak *et al.*[26] were used to evaluate the mutagenic effectiveness and efficiency of the mutagens:

$$\text{Mutagenic effectiveness} = \frac{\text{Rate of mutation (Mp)}}{\text{Duration of treatment} \times \text{concentration}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Rate of mutation (Mp)}}{\text{*Biological damage in } M_1 \text{ generation}}$$

*Biological damage: For measuring the biological damage, three different criteria were used:

- Injury: i.e., percentage reduction in seedling height (Mp/I)
- Sterility: i.e., percentage reduction in pollen fertility (Mp/S)
- Inhibition: i.e., percentage inhibition in seed germination (Mp/R)

RESULTS AND DISCUSSION

Chlorophyll mutation frequency in M_2 generation is one of the most reliable measures for evaluating the mutagen-induced genetic alterations. Based on the intensity of pigmentation at the seedling stage, 5 different types of chlorophyll mutants viz., albina, xantha, chlorina, maculata and virescent were recorded in segregating M_2 plants of lentil (Table 1). Most of the chlorophyll mutant types (albina, xantha and chlorina) were lethal and survived to seedling stage only. Maculata and virescent types however survived upto maturity and produced few seeds. A brief description of different chlorophyll mutants is given below:

- **Albina:** Lethal mutant, white leaves of seedlings, survived for 8-10 days after germination
- **Xantha:** Yellow colored leaves, seedlings survived for 10-15 days only
- **Chlorina:** Light green first pair of leaves in seedlings, emerging leaves were also light green and became darker with the approach of maturity, seedlings died within 15 days
- **Maculata:** Yellow or whitish dots on leaves. Plants were vigorous in growth, late in maturity and produced few seeds
- **Virescent:** White or light yellow colored leaves with patches of yellowish green or green color. Mostly, yellowish or light green patches completely disappeared and the normal color was regained. They were as vigorous as the normal plants and set seeds

Of the five types of chlorophyll mutants recorded in M_2 generation, 'chlorina' followed by 'xantha' types were predominant in all the three mutagens. Joshi-Saha *et al.*[19] reported that electron beam and gamma irradiation induced more number of 'chlorina' mutations in variety vijay of chickpea, while Khan *et al.*[25] reported that chemical mutagens induced high frequency of 'chlorina' mutants in M_2 generation of chickpea. Occurrences of 'chlorina' mutants in large number of crops have been attributed to different causes such as impaired chlorophyll biosynthesis, degradation of chlorophyll and deficiency of carotenoids[31].

Among the mutagens used in the present study, MMS induced the higher frequency and wider spectrum of chlorophyll mutations. The high incidence of chlorophyll mutations induced by MMS, in the present study, may be due

Table 1: Frequency and spectrum of chlorophyll mutants in M_2 generation of lentil

Treatments	No. of M_1 plant progenies	No. of plant progenies segregating in M_2	Percentage of mutated plant progenies (Mp)	No. of M_1 plants	Chlorophyll mutant types: Albina	Xantha	Chlorina	Maculata	Virescent	Total	Frequency (%)
Control	27	-	-	350	-	-	-	-	-	-	-
0.1% MMS	30	12	40.00	295	4	5	9	3	2	23	7.79
0.2% MMS	28	14	50.00	310	6	6	10	2	1	25	8.06
0.3% MMS	31	16	51.61	316	5	7	9	3	3	27	8.54
0.4% MMS	29	17	58.62	325	5	7	11	3	3	29	8.92
Total	-	-	-	-	20	25	39	11	9	104	33.31
0.01% HZ	28	11	39.28	307	4	5	6	1	1	17	5.53
0.02% HZ	29	13	44.82	322	3	5	7	2	2	19	5.90
0.03% HZ	31	14	45.16	330	4	6	7	3	2	22	6.66
0.04% HZ	28	15	53.57	336	5	7	9	2	2	25	7.44
Total	-	-	-	-	16	23	29	8	7	83	25.53
0.01% SA	28	10	35.71	298	4	4	5	1	1	15	5.03
0.02% SA	24	10	41.66	306	3	5	6	2	1	17	5.55
0.03% SA	30	13	43.33	312	3	6	6	1	2	18	5.77
0.04% SA	30	14	46.66	328	3	5	7	3	2	20	6.09
Total	-	-	-	-	13	20	24	7	6	70	22.44

MMS: Methylmethane sulphonate, HZ: Hydrazine hydrate, SA: Sodium azide

Table 2: Effectiveness and efficiency of the mutagens in M_2 generation of lentil

Treatments	% seedling injury (I)	% pollen sterility (S)	% inhibition in seed germination (R)	% mutated plant progenies (Mp)	Mutagenic effectiveness Mp/t.c	Mutagenic efficiency		
						Mp/I	Mp/S	Mp/R
0.1% MMS	20.16	13.42	10.41	40.00	66.66	1.98	2.98	3.84
0.2% MMS	29.03	20.12	14.58	50.00	41.66	1.72	2.48	3.42
0.3% MMS	32.29	24.30	20.83	51.61	28.67	1.59	2.12	2.47
0.4% MMS	38.70	28.20	25.00	58.62	24.42	1.51	2.07	2.34
Average	30.04	21.51	17.70	50.05	40.35	1.70	2.41	3.01
0.01% HZ	17.74	12.90	8.33	39.28	654.66	2.21	3.04	4.71
0.02% HZ	23.38	17.50	12.50	44.82	373.50	1.91	2.56	3.58
0.03% HZ	29.25	20.16	16.66	45.16	250.88	1.54	2.24	2.71
0.04% HZ	36.29	24.20	20.83	53.57	223.20	1.47	2.21	2.57
Average	26.66	18.69	14.58	45.70	375.56	1.78	2.51	3.39
0.01% SA	13.70	10.20	6.25	35.71	595.16	2.60	3.50	5.71
0.02% SA	17.74	15.60	11.45	41.66	347.16	2.34	2.67	3.63
0.03% SA	22.58	18.50	14.58	43.33	240.72	1.91	2.34	2.97
0.04% SA	33.06	23.10	16.66	46.66	194.41	1.41	2.02	2.80
Average	21.77	16.85	12.23	41.84	344.36	2.06	2.63	3.77

MMS: Methylmethane sulphonate, HZ: Hydrazine hydrate, SA: Sodium azide

to its specificity to affect certain regions of chromosomes. Chlorophyll development seems to be controlled by many genes located on several chromosomes which could be adjacent to centromere and proximal segment of chromosome[32-33]. Mutations in these chlorophyll genes are reflected in M_2 and subsequent generations in the form of different types of mutants. A linear relationship between chlorophyll mutations frequency and dose was observed with all the three mutagens (Table 1). Similar dose dependent increase in chlorophyll mutation frequency was reported by Kharkwal[9] and Bara *et al*.[34] in chickpea, Arora and Kaul[35] in pea, John[36] in cowpea, Das and Kundagrami[37] in grasspea, Wani *et al*.[38] in mungbean and Patil and Rane[39] in cluster bean. The frequency of chlorophyll mutations induced by SA was less as compared to MMS and HZ. The low chlorophyll mutations frequency in SA treatments may be due to the inhibition of catalase and peroxidase and an increase in peroxide concentration in the cells[40].

Mutagenic effectiveness and efficiency: Data on effectiveness and efficiency of various mutagenic treatments calculated on the basis of frequency of chlorophyll mutations is given in Table 2. It was found that effectiveness and efficiency were higher at lower concentrations of all the three mutagens used. This is because the lower dose may cause relatively less damage enabling the organism to express the induced gene mutations successfully. The estimates of effectiveness ranged from 24.42-66.66 in MMS treatments, whereas the effectiveness of HZ and SA treatments ranged from 223.20-654.66 and 194.41-595.16, respectively. Mutagenic effectiveness decreased at the highest concentration of all the three mutagens. The order of mutagens based upon effectiveness was HZ>SA>MMS. The HZ was also reported to be an effective mutagen in maize[41], barley[42], sorghum[43] and mungbean[38].

Mutagenic efficiency varied depending upon the criteria selected for its estimation. In this study, the mutagenic efficiency, worked out on the basis of seedling injury (Mp/I), pollen sterility (Mp/S) and inhibition in seed germination (Mp/R), showed a decline with increasing concentrations of all the treatments (Table 2). These results are in conformity to those reported earlier by Khan and Siddiqui[44]. The greater efficiency at lower doses is because the biological damage (seedling injury, pollen sterility and inhibition in seed germination) generally increased with the enhancement in the dose at a higher rate than the mutations yielded in M_2 at the same dose[26]. The mutagenic efficiency was more pronounced in SA treated population than those treated with HZ and MMS. Further, the efficiency calculated on the basis of inhibition in seed germination was higher as compared to those based on pollen sterility and seedling injury. These results are in agreement to the earlier findings of Wani and Khan[45].

CONCLUSION

Improvement in frequency and spectrum of mutations in predictable manner and thereby achieving desired plant characteristics for their direct or indirect exploitation in breeding programmes is an important goal of mutation research. Occurrence of chlorophyll deficient mutants is attributed to change in gene or set of genes responsible for chlorophyll mutations. Mutations in these genes are reflected in M_2 and subsequent generations in the form of different chlorophyll mutants. Chlorophyll mutants though do not have much economic importance due to their lethal nature, yet they could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutants in later generations.

SIGNIFICANCE STATEMENTS

This investigation was aimed to study the frequency of chlorophyll mutations and determine the effectiveness and efficiency of different mutagens in mutant (M_2) generation of lentil. Alteration in the nucleotide sequence of genes controlling the synthesis of enzymes required for biosynthesis of pigments lead to chlorophyll mutations. Chlorophyll mutations are important for identifying gene function and elucidating the chlorophyll metabolism and its regulation. In this study, the chlorophyll mutants induced by different chemical mutagens could be utilized in different mutation breeding programmes for inducing viable mutations of greater economic and agronomic importance.

ACKNOWLEDGMENTS

The author is grateful to the Chairman, Department of Botany, Aligarh Muslim University, Aligarh, India and Principal, Government Degree College (Boys), Anantnag, Jammu and Kashmir, India for providing necessary research and laboratory facilities.

REFERENCES

1. Reddy, A.A. and G.P. Reddy, 2010. Supply side constrains in production of pulses in India: A case study of lentil. Agric. Econ. Res. Rev., 23: 129-136.
2. Lhungdim, J., Y. Singh, S.K. Chongtham and K.N. Devi, 2015. Influence of weed control practices on grain yield and protein content of small seeded lentil (*Lens culinaris* Medikus) under rainfed condition. J. Food Legumes, 28: 249-252.
3. Sharara, F.A., T.A. El-Shahawy and K.G. El-Rokiek, 2011. Effect of prometryn/benzoic acid combination on weeds, seed yield and yield components in lentil (*Lens culinaris* L.). Electron. J. Polish Agric. Univ., 14, No. 1.
4. Awan, M.A., 2005. Mutation Breeding for Crop Improvement: A Review. In: Role of Classical Mutation Breeding in Crop Improvement, Datta, S.K. (Ed.). Daya Publishing House, Delhi, India, pp: 20-35.
5. Gustafsson, A., 1963. Productive mutations induced in barley by ionizing radiations and chemical mutagens. Hereditas, 50: 211-262.
6. Nilan, R.A., 1967. Nature of induced mutations in higher plants. Induced mutations and their utilization. Erwin-Bauer Memorial Lectures IV, Akademie-Verlag, Berlin, Germany, pp: 5-20.
7. Swaminathan, M. S. and N.P. Sarma, 1968. Alteration of the mutation spectrum in barley through treatments of different periods in the S phase of DNA synthesis. Curr. Sci., 37: 685-686.
8. Khalatkar, A.S. and C.R. Bhatia, 1975. Synergistic effect of combined treatments of gamma radiation and ethyl methanesulfonate in barley. Radiat. Bot., 15: 223-229.
9. Kharkwal, M.C., 1998. Induced mutations in chickpea (*Cicer arietinum* L.). II. Frequency and spectrum of chlorophyll mutations. Indian J. Genet. Plant Breed., 58: 465-474.
10. Singh, V.P., M. Singh and J.P. Pal, 1999. Mutagenic effects of gamma rays and EMS on frequency and spectrum of chlorophyll and macromutations in urdbean (*Vigna mungo* L. Hepper). Indian J. Genet., 59: 203-210.
11. Waghmare, V.N. and R.B. Mehra, 2001. Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus* L. Indian J. Genet. Plant Breed., 61: 53-56.
12. Wani, A.A. and M. Anis, 2004. Spectrum and frequency of chlorophyll mutations induced by gamma rays and EMS in *Cicer arietinum* L. J. Cytol. Genet., 5: 143-147.
13. Toker, C. and M.I. Cagirgan, 2004. Spectrum and frequency of induced mutations in chickpea. Int. Chickpea Pigeonpea Newslett., 11: 8-10.
14. Bhat, T.A. and S. Parveen, 2005. Spectrum and frequency of chlorophyll mutations induced by gamma rays and EMS in *Vicia faba* L. Vegetos: Int. J. Plant Res., 18: 143-145.
15. Yamaguchi, H., T. Morishita, K. Degi, A. Tanaka, N. Shikazono and Y. Hase, 2006. Effect of carbon-ion beams irradiation on mutation induction in rice. Plant Mutat. Rep., 1: 25-27.
16. Khan, M.H. and S.D. Tyagi, 2009. Studies on induction of chlorophyll mutations in soybean, *Glycine max* (L.) Merrill. Front. Agric. Chin., 3: 253-258.
17. Arulbalachandran, D. and L. Mullainathan, 2009. Chlorophyll and morphological mutants of black gram (*Vigna mungo* (L.) Hepper) derived by gamma rays and EMS. J. Phytol., 1: 236-241.
18. Girija, M. and D. Dhanavel, 2013. Induced chlorophyll mutation in cowpea (*Vigna unguiculata* L. Walp.). Int. J. Curr. Trends Res., 2: 136-140.
19. Joshi-Saha, A., K.S. Reddy, V.C. Petwal and J. Dwivedi, 2015. Identification of novel mutants through electron beam and gamma irradiation in chickpea (*Cicer arietinum* L.). J. Food Legumes, 28: 99-104.

20. Patial, M., S.R. Thakur, K.P. Singh and A. Thakur, 2017. Frequency and spectrum of chlorophyll mutations and induced variability in ricebean (*Vigna umbellata* Thunb, Ohwi and Ohashi). Legume Res., 40: 39-46.
21. Smith, H.H., 1972. Comparative genetic effects of different physical mutagens in higher plants. In: Induced Mutations and Plant Improvement, International Atomic Energy Agency (IAEA), Vienna, Austria, pp: 75-93.
22. Kumar, R. and S.C. Mani, 1997. Chemical mutagenesis in Manhar variety of rice (*Oryza sativa* L.). Indian J. Genet. Plant Breed., 57: 120-126.
23. Koli, N.R. and K. Ramkrishna, 2002. Frequency and spectrum of induced mutations and mutagenic effectiveness and efficiency in fenugreek (*Trigonella foenumgraecum* L.). Indian J. Genet. Plant Breed., 62: 365-366.
24. Gaikward, N.B. and V.S. Kothekar, 2004. Mutagenic effectiveness and efficiency of ethylmethane sulphonate and sodium azide in lentil (*Lens culinaris* Medik). Indian J. Genet. Plant Breed., 64: 73-74.
25. Khan, S., M.R. Wani, M. Bhat and K. Parveen, 2005. Induced chlorophyll mutations in chickpea (*Cicer arietinum* L.). Int. J. Agric. Biol., 7: 764-767.
26. Konzak, C.F., R.A. Nilan, J. Wagner and R.J. Foster, 1965. Efficient chemical mutagenesis. Rad. Bot., 5: 49-70.
27. Savin, V.N., M.S. Swaminathan and B. Sharma, 1968. Enhancement of chemically-induced mutatin frequency in barley through alteration in the duration of pre-soaking of seeds. Mutat. Res./Fundam. Mol. Mech. Mutagen., 6: 101-107.
28. Nilan, R.A., E.G. Sideris, A. Kleinhofs, C. Sander and C.F. Konzak, 1973. Azide-a poten mutagen. Mutat. Res./Fund. Mol. Mech. Mutagen., 17: 142-144.
29. Kodym, A. and R. Afza, 2003. Physical and chemical mutagenesis. Methods Mol. Biol., 236: 189-203.
30. Gustaffson, A., 1940. The mutation system of chlorophyll apparatus. Lund Univ. Arskr., 36: 1-40.
31. Bevins, M., C.M. Yang and J. Markwell, 1992. Characterization of a chlorophyll-deficient mutant of sweetclover (*Melilotus alba*). Plant Physiol. Biochem., 30: 327-331.
32. Swaminathan, M.S., 1964. Use of induced mutations in plant breeding. J. Sci. Ind. Res., 23: 455-458.
33. Goud, J.V., 1967. Induced mutations in bread wheat. Indian J. Genet., 27: 40-45.
34. Bara, B.M., A.K. Chaurasia and P. Verma, 2017. Gamma rays effect on frequency and spectrum of chlorophyll mutation in chickpea (*Cicer arietinum* L.). J. Pharmacogn. Phytochem., 6: 590-591.
35. Arora, R. and M.L.H. Kaul, 1989. Mutagen-induced chorophyll deficiency in *Pisum sativum*. Cytobios, 57: 189-199.
36. John, S.A., 1999. Mutation frequency and chlorophyll mutations in parents and hybrid of cowpea following gamma irradiation. Indian J. Genet. Plant Breed., 59: 357-361.
37. Das, P.K. and S. Kundagrami, 2000. Frequency and spectrum of chlorophyll mutations in grasspea induced by gamma rays. Indian J. Genet. Plant Breed., 60: 239-241.
38. Wani, M.R., S. Khan and M.I. Kozgar, 2011. Induced chlorophyll mutations. I. Mutagenic effectiveness and efficiency of EMS, HZ and SA in mungbean. Front. Agric. Chin., 5: 514-518.
39. Patil, B.M. and G.M. Rane, 2015. Gamma radiation induced chlorophyll mutations in cluster bean (*Cyamopsis tetragonoloba* (L.) Taub) var. NCB-12. Int. J. Allied Pract. Res. Rev., 2: 75-85.
40. Kleinhofs, A., W.M. Owais and R.A. Nilan, 1978. Azide. Mutat. Res./Rev. Genet. Toxicol., 55: 165-195.
41. Sekhar, V.S.G.C. and G.M. Reddy, 1971. Species locus mutations in maize by chemical mutagens. Curr. Sci., 40: 136-137.
42. Kak, S.N. and B.L. Kaul, 1975. Mutagenic activity of hydrazine and its combinations with maleic hydra-zide and X-rays in barley. Cytobios, 12: 123-128.
43. Reddy, C.S. and J.D. Smith, 1984. Mutagenic effectiveness and efficiency of hydrazine and ethyl methanesulphonate in *Sorghum bicolor*. Indian J. Genet. Plant Breed., 44: 49-54.
44. Khan, S. and B.A. Siddiqui, 1993. Chlorophyll mutations in *Vigna radiata* (L.) wilczek. II Mutagenic effectiveness and efficiency of chemical mutagens. Pak. J. Bot., 25: 161-166.
45. Wani, M.R. and S. Khan, 2003. Chlorophyll mutations in lentil. Trop. Agric., 154: 21-26.