Original Research Article

Ameliorations of Gamma Radiations on Chlorophyll Spectrum and Morphological Characteristics of Peas

Abstract

Aims:we aimed to investigate the chlorophyll spectrum and morphological characteristics of Pea under the influence of gamma radiations.

Methods: The experimental material comprised three pea genotypes viz; Arkel, KashiNandini, and PSM-3. Mature and well-filled seeds were irradiated with 150 and 200 Gy doses of gamma rays, and six treatments with three control were laid into randomized block design at Research Farm, KrishiVigyan Kendra during the spring of 2018-19.

Results: The highest frequency of chlorophyll mutation was recorded in PSM-3 at 150Gy and the lowest in KashiNandini at 200Gy.The widest spectrum of chlorophyll mutation with seven different mutants was recorded in the PSM-3 at a 150 Gy dose. With an increase in the dose of gamma rays, there was a decrease in the mutation frequency in all the varieties.

Conclusions: Gamma rays at a rate of 150 Gywere the best for increasing the mutation frequency and they can be used for the induction of desirable mutations in peas. The obtained results confirm that the high potency of the selected mutagenic doses induced a high phenotypic diversity in the treated population and the isolated distinct mutants were of great economic, as well as academic interest for future breeding on peas. The seeds derived from the control and treated populations should be advanced to further generations, to release more variability for quantitative traits which will favor better selection.

Keywords: Chlorophyll mutants, Gamma Irradiation, Mutation Spectrum, Mutagenic Efficiency

Introduction

Numerous species of legumes have a significant potential to produce high-quality forage for animals, as well as protein-rich food for human consumption. Green pea is an important dietary component because they are low in calories and contain vitamins, minerals, and antioxidants. Among such novel legumes the pea (*Pisumsativum*L.) is quite notable and belongs to the family Leguminosae. A significantly speedier way of improving the crops is mutation breeding. Crop improvement can be easily achieved by irradiating the genotypes using Gamma radiations. To understand the nature and principles behind plant growth and development, mutations are employed as tools and a source of genetically improved crops (Adamu and Aliyu, 2007). Due to its heritable nature mutation breeding is widely used in the breeding program to develop new varieties which are better adapted to environmental stressors. As a powerful physical mutagenesis agent, gamma irradiation has been employed extensively by Kumar and Mishra (2007) and Barela (2022) in peas. Physical mutagens provide handy tools to enhance the natural mutation rate, thereby enlarging the genetic variability and increasing the scope of obtaining desired mutants. Induced mutations can produce a variety of traits that are quantitatively and qualitatively inherited in crops (Malusynski and Ahloowalia, 1995). Irradiation of seeds causes genetic variation and allows plant breeders to select specific genotypes along with improved characteristics says earliness, tolerance, salinity, yield, and quality parameters. An essential goal of mutation research is to increase the frequency and spectrum of mutations in a predictable way and use that information directly or indirectly in breeding programmes. It is possible to use morphological mutations that affect plant components practically and release them as new crop types.Gustaffson (1965), Varghese and Sharma (1994). To obtain a high frequency of the desired mutations in mutation breeding experiments, the choice of an effective and efficient mutagen is crucial. Hence previous knowledge of the effectiveness and efficiency of the use of gamma radiations in some varieties is indispensable to classifying the range of doses of useful mutagens in some breeding programs. So the present investigation was undertaken to study the frequency and spectrum of macro mutations along with the mutagenic effectiveness and efficiency of different doses of gamma rays in the M3 generation of peas (*P. sativum*L.).

Material and methods

The present experiment was carried out at the research farm, College of Agriculture, Gwalior (M.P.). The plant material used was three genotypes of green peas*viz.* Arkel, KashiNandini, and PSM-3 were irradiated with gamma radiation derived from a Cobalt-60 (Co60) source at three doses i.e 0 (Control), 150, and 200Gy. As a physical mutagen gamma irradiation was chosen. Dry seeds of each variety, with a moisture content of 16%, were irradiated with 150 and 200Gy with a radioisotope Co60, Cobalt-60, source (Gamma chamber Model-900 supplied by Bhabha Atomic Research Centre, Mumbai, India) in different doses from 60°C source. Treated seeds of each dose along with the dry and wet control were sown during spring (2018-19) for M1 generation and spring(2019-20) for M2 generation was raised on M1 and M2 plant basis following plant-to-progeny methods in a Randomized Block Design. All the recommended cultural measures namely, irrigation, weeding, and plant protection methods were carried out during the growth period of the crop. At various stages of M2 plant growth, particularly from flowering towards the maturity period, the frequency and spectrum of several types of viable mutants were recorded. A measure of mutagenic efficacy is the frequency of mutations brought on by a given dose of a mutagen. The ratio of factor mutations to biological damage means desirable changes free from associated undesirable changes on mutagenesis. A measure of the proportion of mutations to biological harm is known as mutagenic efficiency. The formula proposed by Konzak and Nilan was used to calculate mutagenic efficiency (1950).

$$Mutagenic\ Efficiency = \frac{Mutation\ Rate\ in\ M2}{Biological\ Damage\ in\ M2\ Generation}$$

While mutagenic efficiency measures the proportion of mutations concerning biological harm, mutagenic effectiveness quantifies the frequency of mutations caused by a unit dose of a mutagen.In order to assess the mutagens' potency as mutagens,Konzak and Nilan's (1965) formula was used.

$$Mutagenic\ Effectiveness = \frac{Mutation\ Rate\ in\ M2}{Gamma\ Rays\ Dose\ in\ Gray\ (Gy)}$$

Mutagenic frequency was calculated as follows:

$$Mutagenic\ Frequency = \frac{No.of\ Mutated\ Plants\ at\ Older\ Stage}{Total\ No.of\ Germinated\ Seedlings} X\ 100$$

Result and discussion

The phenotype may vary to a great degree of variation as a result of any mutational event. The mutations affecting gross morphological changes in plant habit, maturity, pod shape seed color, and seed shape were scored as viable mutations. Such changes in macro mutants have the highest significance in plant breeding because they may sometimes give a desired morphology and phenotype. Induced macro mutants have produced a variety of new commercial cultivars that have demonstrated their value in achieving specific breeding goals. It is also possible to induce new features, which do not exist in the available range of variability in a well-adopted and high-yielding variety. Based on characteristics that researchers repeatedly observed in them throughout their research, these mutants were identified and given names. The frequency and range of various types of viable mutations in the M2 generation are affected by mutagens, as shown in (Table 1). The high frequency of mean mutations in different treatments was found under PSM-3 150Gy (4.30%), while the minimum (1.49%) was under KashiNandini 200Gy dose pre-soaked treatment. Figure 1 showed the graphical representation of the mutation frequency of different doses in all three varieties. According to Gaur and Gaur (2001) investigations in chickpeas, Kartika and Subba (2006) in soybean, and Prem and Gupta (2011) in mustard, it became clear that different physical mutagens induced varied mutation spectra and types of mutants, based not only on the type of mutagen used but also on the variety. In the current study, changes in plant stature, maturity, pod shape, seed colour, and seed shape mutants, as well as viable macro mutations in the pea Arkel variety in the M3 generation, were discovered in gamma-irradiated populationsas reported by Sharma and Kumar (2003) in Bengal gram andBarela (2022) in peas. This might be attributed to the mutagens' diverse modes of action on specific base sequences in multiple genes. The differential frequency and spectrum of viable mutations might be due to the individual impact of the mutagen and its doses employed for the treatments.

In chlorophyll mutations, there was no definite trend within the spectrum of morphological mutations concerning the groups of harm and doses, the chlorophyll mutations scored in the M_2 generation were described and classified following the modified classification of Blixt (1972) as follows: (a) Albina;seedlings emerged completely or nearly white, lethal, and died within two weeks after germination. (b) Xantha: seedlings yellow, light pale yellow, or orange, died within 15 days, and few survived even up to the flowering stage. (c) Chlorina: seedlings emerged greenish yellow and started dying within 15-20 days post-germination, and few survived a little longer without seed setting. (4) Chlorotica: these mutants are lighter in color as compared to the parent variety, viable or semi-viable, producing seeds, but less than in the parent variety.

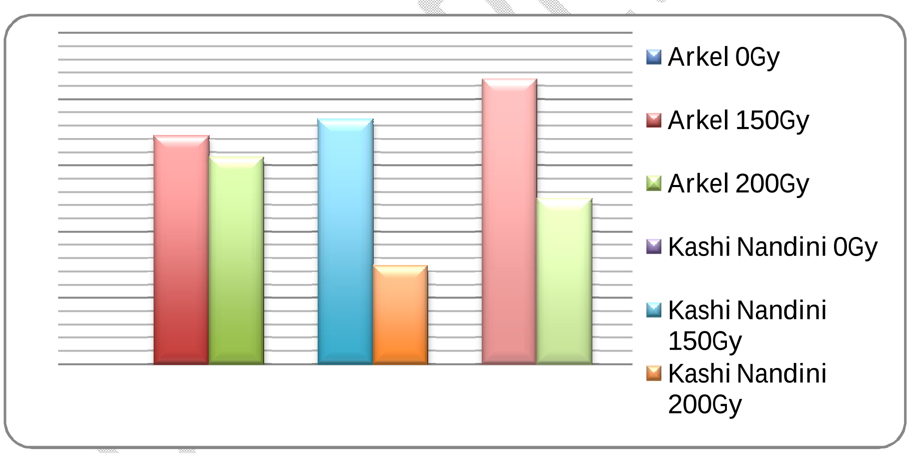

Figure 1: Mutation frequency of different doses of Gamma irradiation in Pea

(5) Viridis: light green, fully viable, and more or less normally productive. (6) Auera: veins colorless and intravenous tissues yellow in color. (7) Variegata: stipules irregularly shaped, with sectors of different colors and sizes. (8) Marginata: Only leaf margins are yellow, inner part of the lamina is green. (9) Vario-maculata: leaves with small and large dark as well as light colored

spots, and sectors of various colors which persist through entire plant life. Irregular darker sectors may also form on testa. (10) Terminalis: green seedlings turning yellow or white after 4-5th node, lethal. Now demonstrated to be mutations for vitamins, and possibly, other essential metabolites.

			
Normal seedling	**Albina**	**Albina green**	**Auera**
			
Auera	**Auera**	**Xantha**	**Clorina**
			

Viridis	Xantha green	Clorina green	Maculata

Figure 2: Chlorophyll mutants showing at the seedling stage of greenpea

Mutation frequency:

All three mutagens (Table 1) used in the present investigation induced a fairly high frequency of chlorophyll mutations, while no mutations were found in the control population. In general, a lower dose (150Gy) of gamma-rays induced a comparatively higher frequency of chlorophyll mutation than a higher dose (200Gy) (Fig.1). Among the different doses, 150Gy treated populations showed maximum chlorophyll mutations (4.30%) followed by (3.70% and 3.45% mutated plants, respectively). Dose-dependent relationship of chlorophyll mutation frequency (Fig. 1) was observed with all three doses. Significant group differences concerning chlorophyll mutation induction were observed with all the mutagens and their doses. The highest frequency of chlorophyll mutation was recorded in the PSM-3 at 150Gy ' and the lowest in KashiNandini at 200Gy. KashiNandini at 150Gy showed higher mutants in Arkel at 150Gy followed by 200Gy and PSM-3 at 200Gy dose concerningthe frequency of chlorophyll mutation. Similar results were found by Barela (2022) in peas and Wani (2009) in chickpeas.

Mutation spectrum:

Lower doses induced a wider spectrum of chlorophyll mutations (Table 1) than the higher doses of radiation. Among the lower dose, PSM-3 showed the widest spectrum with seven types of mutation. The following widest spectrum of chlorophyll mutation was obtained with a lower dose of KashiNandini, inducing six out of nine mutation types. Arkel at 150Gy dose induced only single types of chlorophyll mutation and PSM-3 at 200Gy induced two types of chlorophyll mutation. Among the various chlorophyll mutations, some were induced more frequently: Chlorina 25.9%, Viridis 22.2%, Xantha 14.81%, and Costata11.11% of all chlorophyll mutations (Figure 3). Similarly Mutation breeding has been successfully used for crop development as well as to support efforts made using conventional plant breeding techniques. Other than traditional plant breeding techniques, induced mutation has made it simple to change genesBarela (2019). At the same time, Chlorina and Viridis appeared more frequently with gammarays under lower doses. This showed preferential induction of certain mutations with some mutagens than with others, although only in quantitative terms.

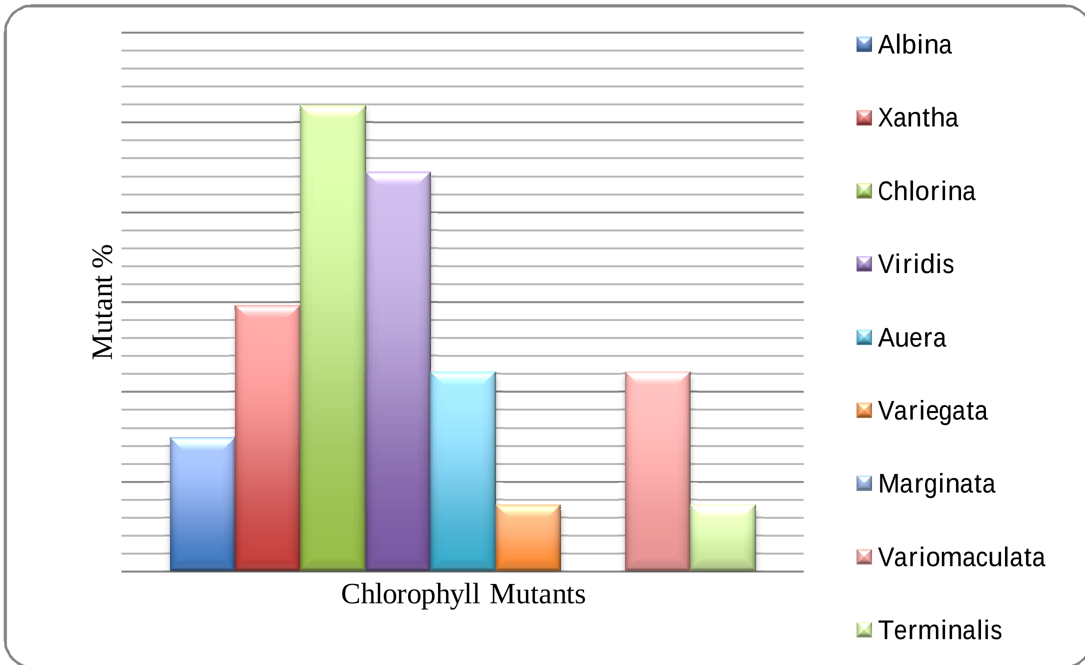

Figure 2 Frequency of different chlorophyll mutants

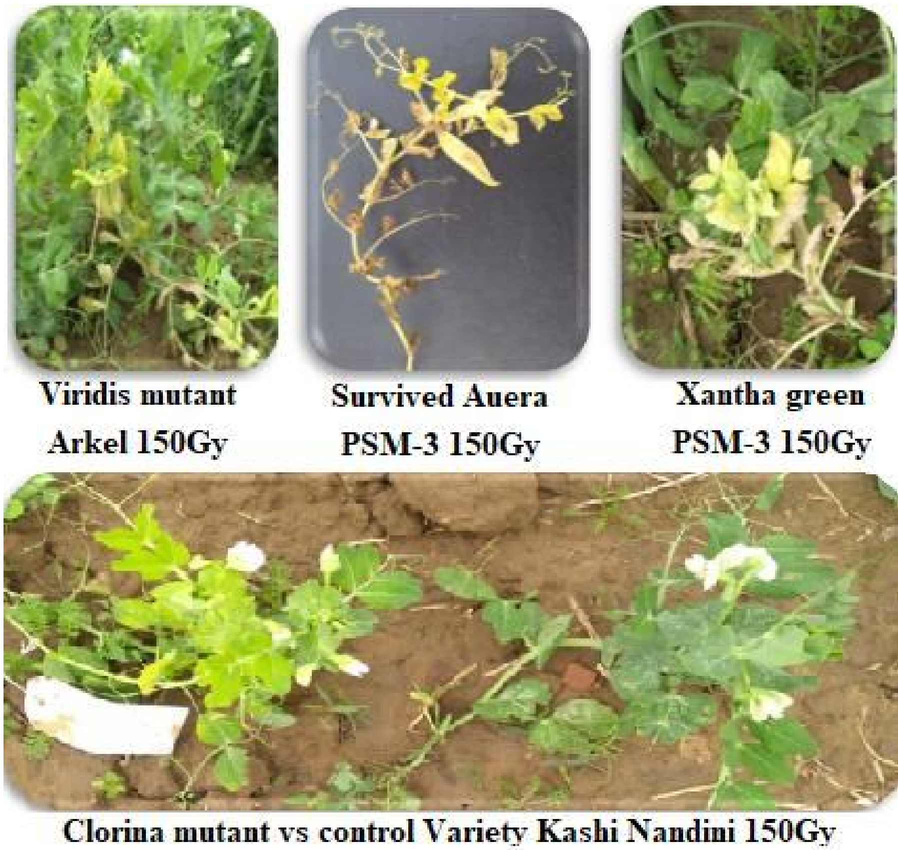

Figure 4: Photograph of plant varieties

Table 1: Frequency of chlorophyll mutants in M2 generation

Variety	**Arkel**			**KashiNandini**			**PSM-3**			**Different Mutant (%)**
Doses	0Gy	150Gy	200Gy	0Gy	150Gy	200Gy	0Gy	150Gy	200Gy	
Total Plant	120	145	96	120	108	67	120	256	120	
Albina	0	0	1	0	0	0	0	0	1	7.41
Xantha	0	1	0	0	0	0	0	3	0	14.81
Chlorina	0	1	1	0	2	0	0	2	1	25.93

Viridis	0	2	0	0	1	1	0	2	0	22.22
Auera	0	0	1	0	1	0	0	1	0	11.11
Variegata	0	0	0	0	0	0	0	1	0	3.70
Marginata	0	0	0	0	0	0	0	0	0	0.00
Variomaculata	0	1	0	0	0	0	0	1	1	11.11
Terminalis	0	0	0	0	0	0	0	1	0	3.70
Mutants (%)	0	3.45	3.13	0.0	3.70	1.49	0.00	4.30	2.50	

Mutagenic effectiveness

Mutagenic effectiveness means the rate of mutation induction is dependent upon the mutagenic doses (Nilan and Fonzak, 1965). It is an index of genotypic response to various doses of a mutagen. The effectiveness of the mutagens tested in the present study differed considerably. The mutagenic effectiveness of PSM-3 at 150Gy (20.83%) was followed by KashiNandini at 150Gy (19.4%). Thus, a lower dose of gammarays was much more effective than higher doses. Similar results were recorded by Dhanavel and Pavadai (2008) in black gram, Khan and Tyagi (2010) in Soybean, and Kumar and Mishra (2016) in peas.

Mutagenic efficiency

The mutagenic efficiency gives an idea of the proportion of mutations concerning other associated undesirable biological effects such as injury, lethality, and sterility induced by the mutagen (Konzak and Nilan, 1965). Mutagenic efficiency was calculated based on pollen sterility and lethality or plant survival percent. Mutagenic efficiency is calculated based on both the percentage of pollen sterility and plant survival. The results showed that all the mutagens used have significantly different mutagenic efficiency. Among the mutagens tested, lower dose of gamma-radiations was the best mutagen (150Gy), followed whereas the (0Gy) dose was the least efficient. Similar results have been reported by Jabee and Ansari (2009) in chickpeas,Wani (2009) in chickpeas, andKhursheed and Laskar (2015) in Viciafeba and Barela (2022). Mutagenic effectiveness and efficiency have much importance in mutation breeding experiments. The response of genotype to the increasing doses of mutagen represents the mutagenic effectiveness and efficiency represents the proportion of mutations concerning the undesirable biological effects. Mutagenic effectiveness and efficiency were observed in the M3 generation. The morphological mutants identified in the present study may not be economically feasible to commercialize directly due to the presence of some undesirable traits but in the context of the plant breeders, these can be further exploited as a source of many elite genes or as parents in hybridization programs.

Conclusion

For inducing desirable mutations in peas, gamma rays at a rate of 150 Gy were the most effective at raising the mutation frequency. According to the results, the high potency of the chosen mutagenic doses caused a high level of phenotypic diversity in the treated population, and the isolated distinct mutants were of enormous economic and intellectual interest for pea breeding in the future. To release more variability for quantitative traits that will favor better selection, the seeds from the control and treated populations should be advanced to subsequent generations.

References

Adamu AK, Aliyu H. Morphological effects of sodium azide on tomato (*Lycopersiconesculentum* Mill). Sci. World J. 2007; 2:9- 12.

Barela A, Gupta N, Jain S, Tiwari S and Kandelkar VS.Current advancement and future appraisal of mutation breeding for genetic improvement of crop.International Journal of Advance and Innovative Research 2019; Volume 6, Issue 1: 67-70.

Barela A, Jain S, Tiwari S, Rahangdale S, Singh P. Impact of gamma radiations on seed germination and morphological characteristics of pea (*Pisumsativum* L.)The Pharma Innovation Journal 2022; 11(8): 464-467.

Blixt (1972) Mutation genetics in PisumAgriHortiqueGenetica 30, 1-293.

Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M, Thilagavathi C. Effectiveness and efficiency of chemical mutagens in cowpea (*Vignaunguiculata* (L.) Walp).Afr J Biotechnol. 2008;7(22):4116–4117.

Gaur PM, Gour VK.A gene inhibiting flower colour in chickpea (*Cicerarietinum* L.).Indian J. Genet. 2001;62(3):273-274.

Gustaffson A. Characterization and role of highly productive mutations in diploid barley. Rad. Bot. 1996;5(Suppl.):323-337.

Jabee F, Ansari MYK.Mutagenic effectiveness and efficiency of hydrazine sulphate (HS) in inducing cytomorphological mutation in Cicerarietinum L. var. K. 850. J. Cytol. Genet. 2005;6(2):161-166.

Kartika R, Subba Lakshmi B. Effects of gamma-rays and EMS on two varieties of soybean. Asian J. Plant Sci. 2006;5(4):721-724.

Khan MH, Tyagi SD. Studies on effectiveness and efficiency of gamma rays, EMS and their combination in soybean [Glycine max (L.) Merrill.]. J Plant Breed Crop Sci. 2010;2(3):055–58.

Khursheed S, Laskar RA, Raina A, Amin R, Khan S. Comparative analysis of cytological abnormalities induced in Viciafaba L. genotypes using physical and chemical mutagenesis. Chromosome Sci. 2015;18(3–4):47–51.

Konzak CF, Bottino PJ, Nilan RA, and Coxger BV. In Neutron Irradiation of Seeds II . Vienna, IAEA Technical Report Series No.(63)MACFARLANE, EILEEN, W.E. Genetics.1950;(35):122–123.

Konzak CF, Nilan RA, FroeseGerten EE, Foster RJ. Factors affecting the biological action of mutagens. In: Proc. Symposium on Induction of Mutation and the Mutation Process, Prague. 1965;123-132.

Kumar A, Chaurasia AK, Marker S, Shukla PK, Rai PK, Verma PK, Bara BM. Effect of gamma radiation of macro mutations, effectiveness and efficiency under M2 generation in pea (*Pisumsativum*L.). Annals of West University of Timişoara, Ser. Biology. 2016;71-76.

Kumar A, Mishra MN, Kharkwal MC. Induced mutagenesis in black gram (Vignamungo L. Hepper).Indian Journal of Genetics. 2007;67(1):41-46.

Malusynski M, Ahloowalia BS, Sigurbjornsson BS. Application of in vivo and in vitro mutation techniques for crop improvement.Euphytica. 1995;85:303-315.

Nilan RA, Fonzak CF, Wagner J, Legault RR. Effectiveness and efficiency of radiation for inducing genetic and cytogenetic changes. Rad. Bot. 1965;5:71- 89.

Prem D, Gupta K, Agnihotri A. Can we predict mutagen induced damage in plant systems mathematically? Insights from zygotic embryo and haploid mutagenesis in Indian mustard (*Brassica juncea*).BotanicaSerbica. 2011;35:137-143.

Sharma V, Kumar G. EMS induced viable macro mutants in *Cicerarietinum* L. J. Cytol. Genet. 2003;4:85-89.

Varghese G, Sharma B. Changes in protein quantity and quality associated with a mutation for amber grain colour in wheat. Sci. 1994;35:469-470.

Wani AA. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combination treatments in chickpea (*Cicerarietinum* L.). Asian J Plant Sci. 2009;8(4):318.