

Original Research Article

Ameliorations of Gamma Radiations Effectiveness on Chlorophyll Spectrum and Morphological Characteristics of Peas (*Pisum sativum L.*)

Abstract

Aims: ~~we aimed to investigate the Chlorophyll chlorophyll Spectrum spectrum and Morphological morphological Characteristics characteristics of Pea under the influence of gamma radiations.~~

Methods: The experimental material comprised ~~of~~ three pea genotypes viz; Arkel, KashiNandini, and PSM-3. Mature and ~~well-well-~~filled seeds ~~of these genotypes~~ 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, Krishi Vigyan Kendra during ~~the Rabi spring of~~ 2018-19.

Results: ~~In this investigation, t~~he 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 ~~were was~~ recorded in ~~the~~ PSM-3 ~~variety~~ 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 Gy is concluded that 150 Gy dose of gamma rays was the best for increasing the mutation frequency and it 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 favour 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 several vitamins, minerals, and antioxidants. Among such novel legumes the pea (*Pisum sativum* 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 with Gamma radiations. To understand the nature and principles behind plant growth and development, mutations are employed as tools and as 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 the new and varieties which are better adaptive

adapted to ~~the changing~~ environmental stressors and an important source of variation creation in plants. 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 ~~have the ability to can quickly~~ produce a variety in 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. Improvement in the frequency and spectrum of mutations in a predictable manner and thereby direct or indirect exploitation in the breeding programme is an important goal of mutation research. ~~Morphological~~ A morphological mutation affecting plant parts can be utilized practically and ~~can be~~ released directly as crop varieties 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~~ the gamma radiations in a number of some varieties is indispensable to classifying the range of doses of useful mutagens in a number of some breeding programmes. 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 ~~investigated~~ 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 ~~3-three~~ doses i.e 0 Gy (Control), 150Gy, 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 ~~of gamma rays~~ with a radioisotope Co60, Cobalt-60, source (Gamma chamber Model-900 supplied by Bhabha Atomic Research Centre, Mumbai, India) in different ~~of~~ doses from 60°C source. Treated seeds of each doses along with the dry and wet control were sown during ~~rabi-spring~~ (2018-19) for M1 generation and ~~rabispring~~ (2019-20) for M2 generation was raised on M1 and M2 plant basis following plant ~~to-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. Mutagenic efficiency was calculated by the formula suggested by Konzak and Nilan (1950).

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

While mutagenic efficiency measures the proportion of mutations ~~in relation to concerning~~ biological harm, mutagenic effectiveness quantifies the frequency of mutations caused by a unit dose of a mutagen. ~~Formula suggested by~~ Konzak and Nilan's (1965) ~~formula were was~~ used to evaluate the mutagenic effectiveness and efficiency of the mutagens used.

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

Mutagenic frequency was calculated ~~by using following formula~~ as follows:

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

Result and discussion

The phenotype may vary to a great degree of variation ~~significantly or only little~~ as a result of any mutational event. The mutations affecting gross morphological changes in plant habit, maturity, pod shape seed colour, 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 well-adopted and high high-yielding variety. These mutants were identified and given names based on ~~distinctive~~ traits that researchers repeatedly saw in them throughout ~~the course of~~ their research. Table 1 ~~shows~~ showed how mutagens affect the frequency and range of various types of viable mutations in the M2 generation. The high frequency of mean mutations in different treatments was found under PSM-3 150Gy ~~dose~~ (4.30%), while the minimum (1.49%) was under KashiNandini 200Gy dose pre-soaked treatment. Figure 1 ~~shows~~ showed the graphical representation of the mutation frequency of different doses in all three varieties. According to ~~earlier investigations~~ Gaur and Gaur (2001) investigations in chickpeas, Kartika and Subba (2006) in soybean, and Prem and Gupta (2011) in mustard, it was evident that

different mutation spectra and types of mutants were caused by different physical mutagens, depending not only on the type of mutagen utilized but also on the variety. In the present investigation, viable macro mutations in the pea Arkel variety in the M3 generation along with changes in attributes characters like plant stature, maturity, pod shape, seed color, and seed shape mutants were recorded in gamma-gamma-irradiated population as reported by Sharma and Kumar (2003) in Bengal-Bengal gram and Barela (2022) in peas. This might be as a result of 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 in relation to concerning the groups of harm and doses, the chlorophyll mutations scored in the M₂ generation were described and classified in accordance with following

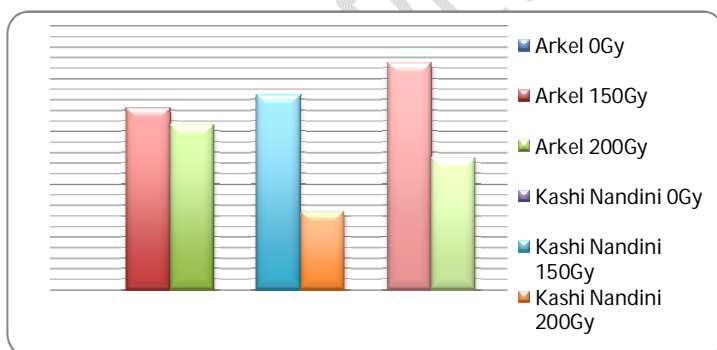


Figure 1: Mutation frequency of different doses of Gamma irradiation in Pea

the modified classification of Blixt (1972) as follows:- (a)

i. ——— Albina: Seedlings emerged completely or nearly white, lethal, and died within two weeks after germination. (b)

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ii. —Xantha: ~~Seedlings~~ seedlings yellow, light pale yellow, or orange, died within 15 days, and few survived even up to the flowering stage. (c)

iii. —Chlorina: ~~Seedlings~~ seedlings emerged greenish yellow and started dying within 15-20 days ~~after~~ post-germination, and few survived a little longer without seed setting. (4)

iv. Chlorotica: ~~These~~ 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.

v. —(5) Viridis: ~~Light~~ light green, fully viable, and more or less normally productive. (6)

vi. —Aurea: ~~Veins~~ veins colorless and intravenous tissues yellow in color. (7)

vii. —Variegata: ~~Stipules~~ stipules irregularly shaped, with sectors of different colors and sizes. (8)

viii. —Marginata: Only leaf margins are yellow, inner part of the lamina is green. (9)

ix. —Vario-maculata: ~~Leaves~~ 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)

x. Terminalis: ~~Green~~ green seedlings turning yellow or white after 4-5th node, lethal. Now demonstrated to be mutations for vitamins, and possibly, other essential metabolites.

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Normal seedling

Albina

Albina green

Aurea

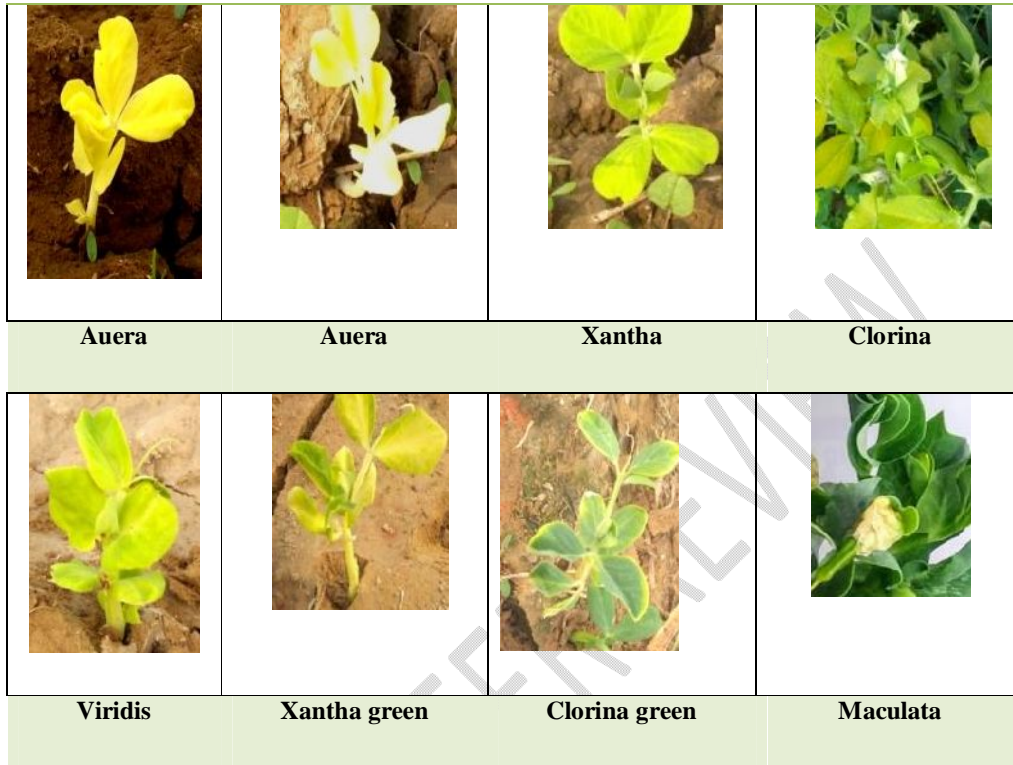


Figure 2: Chlorophyll mutants showing at [the](#) seedling stage of greenpea

Mutation frequency:

~~The detailed results of chlorophyll mutation frequency induced by various mutagens are presented in table 1.~~ 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 ~~untreated~~ (control) population. In general, a lower dose (150Gy) of gamma-rays induced a comparatively higher frequency of chlorophyll mutation than a higher dose (200Gy) ~~gamma irradiation~~ (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. ~~The highest chlorophyll mutation frequency (4.30% mutants) was recorded with the lower dose of 150Gy and the lowest frequency (1.49% mutants) with the higher dose (200Gy) of gamma rays.~~ Significant group differences ~~with regard to 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 ~~gamma rays~~ showed higher mutants in Arkel at 150Gy followed by 200Gy and PSM-3 at 200Gy dose ~~with regard to concerning the~~ frequency of chlorophyll mutation. Similar results were found by Barela (2022) in peas and Wani (2009) in chickpeas.

Mutation spectrum:

~~The relative spectrum of chlorophyll mutation is summarized in table 1. It is evident that il~~ Lower doses induced a wider spectrum of chlorophyll mutations (Table 1) than ~~the~~ higher doses of radiation. Among the lower dose, ~~in~~ PSM-3 showed the widest spectrum with ~~7-seven~~ types of mutation ~~appearing in its treatments~~. The ~~next following~~ widest spectrum of chlorophyll mutation was obtained with ~~a~~ lower dose of KashiNandini, inducing ~~6-six~~ out of ~~9-nine~~ mutation types. Arkel at 150Gy dose ~~of gamma ray~~ induced only single types of chlorophyll mutation and PSM-3 at 200Gy induced ~~2-two~~ types of chlorophyll mutation. Among the various chlorophyll mutations, some were induced more frequently: ~~chlorina-Chlorina~~ 25.9% ~~per cent~~, ~~viridis~~ Viridis 22.2% ~~per cent~~, ~~xantha-Xantha~~ 14.81% ~~per cent~~ and ~~costata~~ Costata 11.11% ~~per cent~~ of all ~~the~~ chlorophyll mutations (figure ~~Figure~~ 3). At the same time, ~~chlorina-Chlorina~~ and ~~viridis~~ Viridis appeared more frequently with gamma-rays under lower doses. This ~~shows~~ showed preferential induction of certain mutations with some mutagens than with others, although only in quantitative terms.

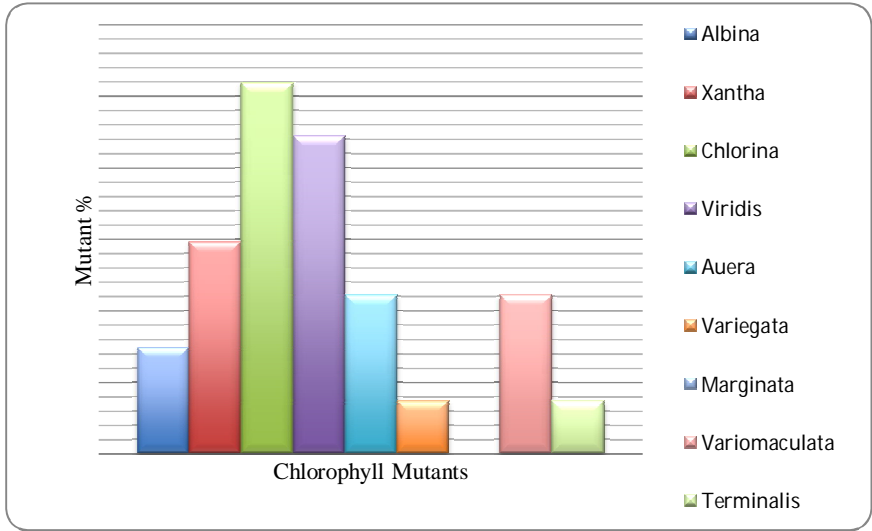


Figure 23 Frequency of different chlorophyll mutants in pea

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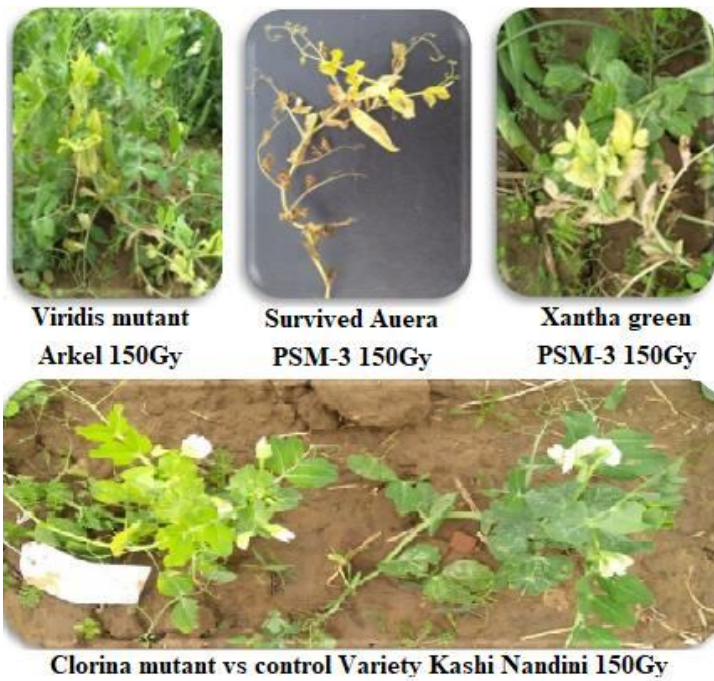


Figure 4: Photograph of plant varieties

Table 1: Frequency of chlorophyll mutants in M2 generation

Variety	Arkel			KashiNandini			PSM-3			Different Mutant (%)
	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 ~~as 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. ~~Among the different doses used, lower dose were found to be more effective than higher dose.~~ The mutagenic effectiveness of PSM-3 at 150Gy (20.83%) ~~was~~ followed by KashiNandini at 150Gy (19.4%). Thus, ~~a~~ lower dose of gamma-rays was much ~~more~~ effective ~~than~~ higher doses ~~of gamma rays~~. 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 ~~in relation to concerning~~ other associated undesirable biological effects such as injury, lethality, and sterility induced by the mutagen. (Konzak and Nilan, (1965). Mutagenic efficiency was calculated ~~on the basis~~ ~~based on~~ pollen sterility and lethality or plant survival percent. Mutagenic efficiency ~~is~~

calculated ~~on the basis of~~based on both ~~the~~ percentage of pollen sterility and ~~percentage~~ 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 ~~of gamma irradiation~~ was the least efficient. Similar results have been reported by Jabee and Ansari (2009) in chickpeas~~s~~, Wani (2009) in chickpeas~~s~~, ~~and~~ Khursheed and Laskar (2015) in Vicia faba and Barela (2022), (Table 2). 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 ~~in relation to~~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 ~~a~~ parents in hybridization programmes.

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