__Original Research Article__

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

Abstract

Aims: Chlorophyll Spectrum and Morphological Characteristics of Pea

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

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

Conclusions: It is concluded that150 Gy dose of gamma rays was the best for increasing the mutation frequency and it can be used for induction of desirable mutations in pea. The obtained results confirms 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 pea. The seeds derived from control and treated population should be advanced to further generations, to release more variability for quantitative traits which will favour better selection.

Keyword: 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 people. Green pea is an important dietary component because they are fairly 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 family Leguminosae. A significantly speedier way of improving the crops is mutation breeding. Crop improvement can be easily achieved by irradiating the genotypes with the help of Gamma radiations. In order 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 breeding program to develop the new and varieties which are better adaptive to the changing environment and an important source of variation creation in plants. As a powerful physical mutagenesis agent, gamma irradiation has been employed extensively Kumar and Mishra (2007) and Barela (2022) in pea. Physical mutagens provide handy tools to enhance natural mutation rate, thereby enlarging the genetic variability and increasing the scope of obtaining desired mutants. Induced mutations have the ability to quickly produce variety in traits that are quantitatively and qualitatively inherited in crops Malusynski and Ahloowalia (1995). Irradiation of seeds causes genetic variation 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 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 effectiveness and efficiency of used to the gamma radiations in a number of varieties is indispensable to classify the range of doses of useful mutagens in a number of 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 M3 generation.

Material and methods

The present experiment was investigated at the research farm, College of Agriculture, Gwalior (M.P.). The plant material used was three genotypes of green pea *viz*. Arkel, Kashi Nandini and PSM-3 were irradiated with gamma radiation derived from Co60 source at 3 doses i.e 0 Gy (Control), 150Gy and 200Gy. As a physical mutagen gamma irradiation was chosen. Dry seeds of each variety, with moisture content 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* (2018-19) for M1 generation and *rabi* (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 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).

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

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

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

Mutagenic frequency was calculated by using following formula –

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

Result and discussion

The phenotype may vary 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 change in macro mutants have 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. 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 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 Kashi Nandini 200Gy dose pre-soaked treatment. Figure 1 shows the graphical representation of mutation frequency of different doses in all three varieties. According to earlier investigations Gaur and Gaur (2001) in chickpea, Kartika and Subba (2006) in soybean, 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 pea Arkel variety in M3 generation along with changes in attributes characters like plant stature, maturity, pod shape, seed colour and seed shape mutants were recorded in gamma irradiated population as reported by Sharma and Kumar (2003) in bengal gram and Barela (2022) in pea. This might be as a result of 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 the groups of harm and doses, the chlorophyll mutations scored in M_2 generation were described and classified in accordance with the modified classification of Blixt (1972).

i. Albina: Seedlings emerged completely or nearly white, lethal, died within two weeks after germination.

ii. Xantha: Seedlings yellow, light pale yellow, or orange, died within 15 days, few survived even up to flowering stage.

iii. Chlorina: Seedlings emerged greenish yellow and started dying within 15-20 days after germination, few survived a little longer without seed setting.

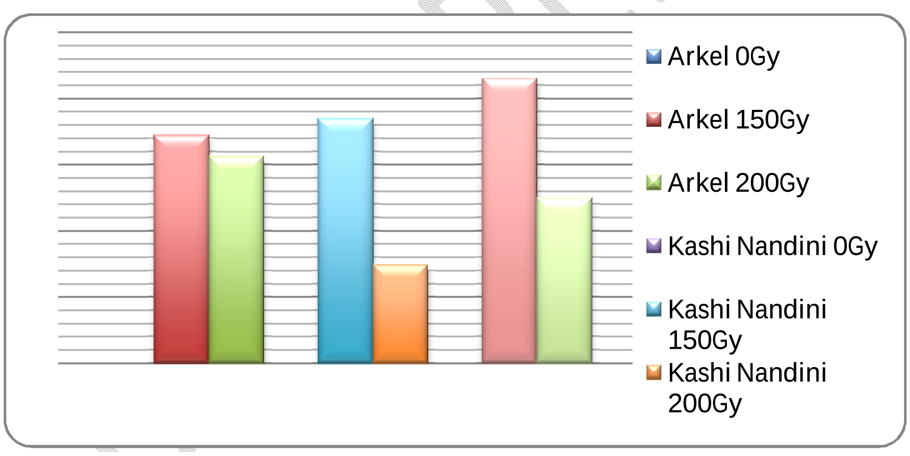

Figure 1: Mutation frequency of different doses of Gamma irradiation in Pea

iv. 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.

v. Viridis: Light green, fully viable and more or less normally productive.

vi. Auera: Veins colorless and intravenous tissues yellow in color.

vii. Variegata: Stipules irregular shaped, with sectors of different color and size.

viii. Marginata: Only leaf margins yellow, inner part of lamina green.

ix. Vario-maculata: Leaves with small and large dark as well as light colored spots, and sectors of various color which persist through entire plant life. Irregular darker sectors may also form on testa.

x. 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**

Figure 2: Chlorophyll mutants showing at 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 used in the present investigation induced fairly high frequency of chlorophyll mutations, while no mutations were found in the untreated (control) population. In general, lower dose (150Gy) of gamma-rays induced comparatively higher frequency of chlorophyll mutation than 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 150Gyand the lowest frequency (1.49% mutants) with the higher dose (200Gy) of gamma-rays. Significant group differences with regard to chlorophyll mutation induction were observed with all the mutagens and their doses. The highest frequency of chlorophyll mutation was recorded in PSM-3 at 150Gy ' and the lowest in Kashi Nandini at 200Gy. Kashi Nandini at 150Gy gamma-rays showed higher mutants in Arkel at 150Gy followed by 200Gy and PSM-3 at 200Gy dose with regard to frequency of chlorophyll mutation. Similar results were found by Barela (2022) in pea and Wani (2009) in chickpea.

Mutation spectrum:

The relative spectrum of chlorophyll mutation is summarized in table 1. It is evident that lower dose induced a wider spectrum of chlorophyll mutations than higher dose of radiation. Among the lower dose in PSM-3 showed the widest spectrum with 7 types of mutation appearing in its treatments. The next widest spectrum of chlorophyll mutation was obtained with lower dose of Kashi Nandini, inducing 6 out of 9 mutation types. Arkel at 150Gy dose of gamma-ray induced only single types of chlorophyll mutation and PSM-3 at 200Gy induced 2 type of chlorophyll mutation. Among the various chlorophyll mutations, some were induced more frequently: chlorina 25.9 per cent, viridis 22.2 per cent, xantha 14.81 per cent and costata11.11 per cent of all the chlorophyll mutations (figure 3). At the same time, chlorina and viridis appeared more frequently with gamma-rays under lower dose. This shows preferential induction of certain mutations with some mutagens than with others, although only in quantitative terms.

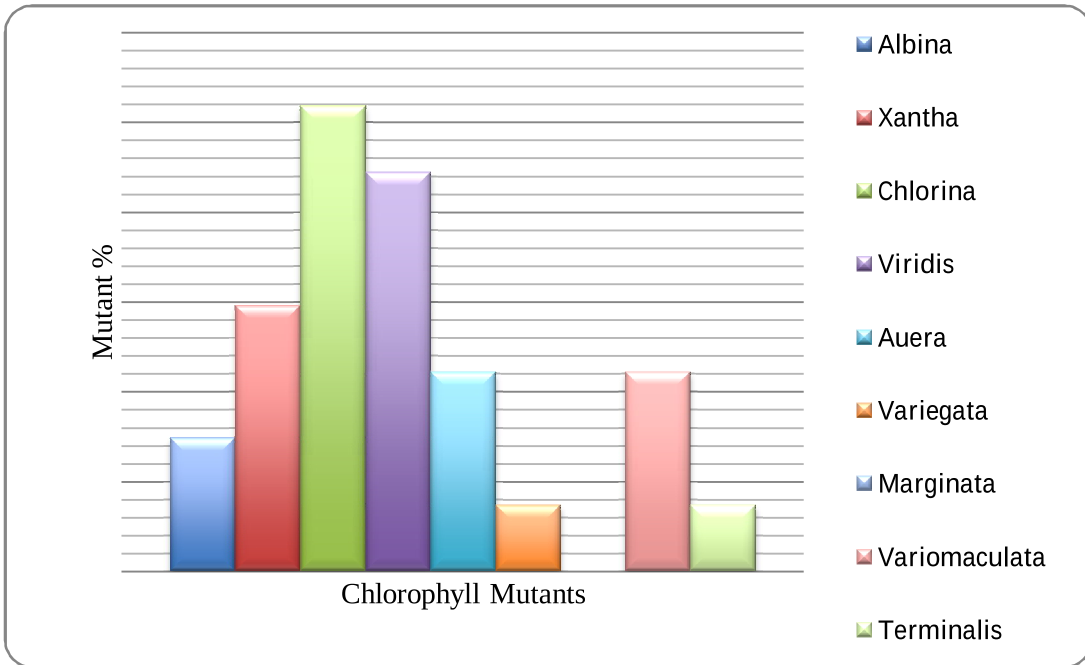

Figure 2 Frequency of different chlorophyll mutants in pea

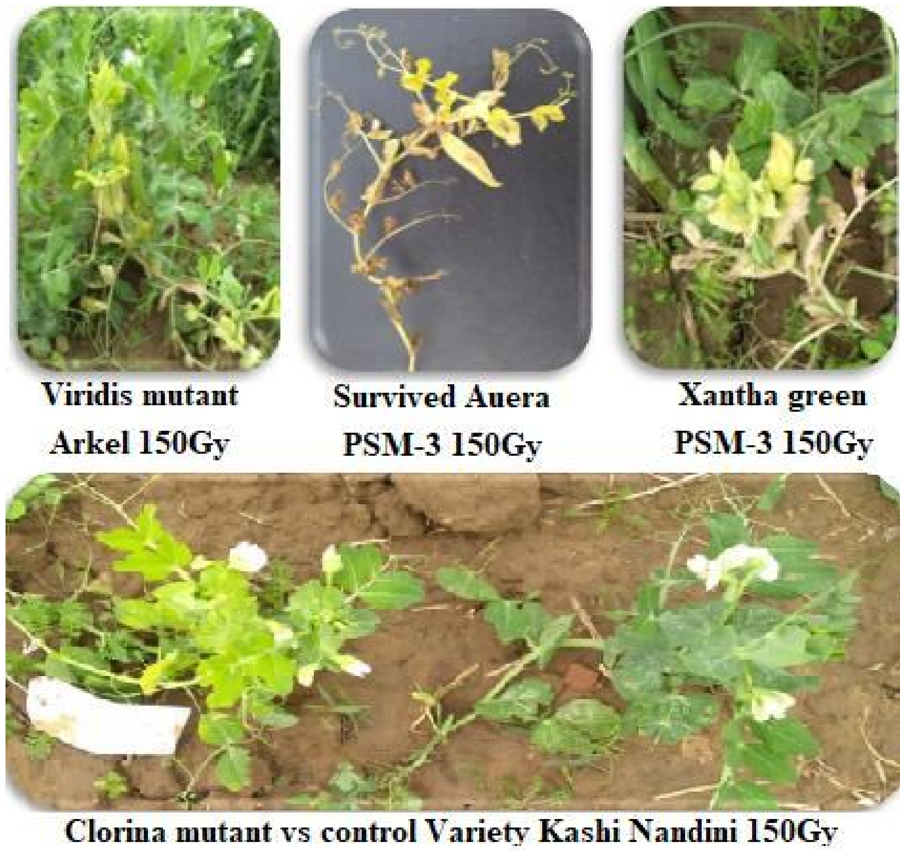

Figure 4: Photograph of plant varieties

Table 1: Frequency of chlorophyll mutants in M2 generation

Variety	**Arkel**			**Kashi Nandini**			**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 as 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%) followed by Kashi Nandini at 150Gy (19.4%). Thus, lower dose of gamma-rays was much effective 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 pea.

Mutagenic efficiency

The mutagenic efficiency gives an idea of the proportion of mutations in relation to 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 of pollen sterility and lethality or plant survival percent. Mutagenic efficiency calculated on the basis of both percentage 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 best mutagen (150Gy), followed whereas (0Gy) dose of gamma irradiation was the least efficient. Similar results have been reported by Jabee and Ansari (2009) in chickpea; Wani (2009) in chickpea; Khursheed and Laskar (2015) in Viciafeba 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 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 context of the plant breeders, these can be further exploited as source of many elite genes or as a parents in hybridization programmes.

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