



Mutagenic Effect of Gamma Radiation on Macro Mutation Spectrums, Effectiveness and Efficiency under M₃ Generation in Pea (*Pisum sativum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mutation breeding is one of the most reliable techniques in improving crop plants. The study was frequency and spectrum of macro-mutations along with the mutagenic effectiveness and efficiency of different doses of gamma rays in pea variety (Arkel). The irradiated seeds were sown in the M₁, M₂ generations in their respective control and harvested in bulk to raise the M₃ generation (2013-14) in Randomized Block Design with three replications. Screening of the M₃ generation revealed that the mutagenic treatments induced morphological and physiological changes in the variety. A spectrum of mutations which included variants with respect to plant stature, maturity, pod shape, seed colour and seed shape were observed mutagenic effectiveness is measure of the frequency of mutations induced by unit mutagen doses whereas mutagenic efficiency is measure of proportion of mutation in relation to undesirable changes like lethality and sterility etc. The frequency of mutagenic efficiency and effectiveness was found to be highest at lower doses. In mutation

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breeding where large populations are handled, estimation of mutagenic effect for macro mutation, effectiveness and efficiency may help the breeders in identifying effective treated populations in early generation for reduction in cost of breeding and enhancing scope of selection.

Keywords: Gamma radiation; pea; spectrum of macro mutation; effectiveness; efficiency.

1. INTRODUCTION

A large number of legume species possess great potential for contributing to not only protein-rich food for humans but also excellent quality forage for animals. Among such novel legumes the pea (*Pisum sativum* L.) is quite notable and belongs to family Leguminosea. Mutation breeding is relatively a quicker method improvement the crops. Mutations are the tools and being used to study the nature and basis of plant growth and development thereby producing raw materials for genetic improvement of crops [1]. Gamma irradiation has been used extensively as a potent physical mutagenic agent [2]. 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 can rapidly create variability in quantitatively and qualitatively inherited traits in crops [3]. 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 of immense practical utility and many of them have been released directly as crop varieties [4,5]. Selection of effective and efficient mutagen is very essential to recover high frequency of the desirable mutations in a mutation breeding studies. 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.

2. MATERIALS AND METHODS

The present research was conducted in the Field Experimental Centre, Department of Genetics and Plant Breeding at Sam Higginbottom Institute of Agriculture, Technology and

Sciences, Allahabad (U.P.) India. The plant material used was the Arkel variety of pea and irradiated with physical mutagen viz. gamma rays for 00kR (dry seeds control), 05kR (dry seeds), 10kR (dry seeds), 15kR (dry seeds), 20kR (dry seeds), 25kR (dry seeds), 30kR (dry seeds), 35kR (dry seeds) 40kR (dry seeds) and pre-soaked seeds of the same was exposed to 00kR (wet seeds), 05kR (wet seeds), 10kR (wet seeds), 15kR (wet seeds) 20kR (wet seeds) (kilo Roentgen) in different of doses from 60°C source at National Botanical of Research Institute, Lucknow (U.P.). Treated seeds of each doses along with the dry and wet control were sown during the rabi season (2011-12) for raising the M₁ generation. Field trails was laid out M₃ generation was raised on M₁ and M₂ plant basis following plant to progeny methods in a Randomized Block Design with three replications during the month of November in the year (2013-14). All the recommended cultural measures namely, irrigation, weeding and plant protection methods were carried out during the growth period of the crop. The frequency and spectrum of different types of viable mutants were scored at various developmental stages of M₃ plants particularly from flowering to maturity period. These mutants were classified for deviation from the normal and taking into consideration the most important characters namely plant stature (tall, dwarf and semi dwarf), maturity (early and late), pod shape (bold, long and short), seed colour (brown, light white and light green) and seed shape (small, bold and wrinkled) in life period of the plants. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of a mutagen. Formulate suggested by [6] were used to evaluate the mutagenic effectiveness of the mutagens used.

Mutagenic effectiveness =

$$\frac{\text{Mutation rate on the basis of M1 plant progenies (MP)}}{\text{M3 population (MS) Dose in kilo Roentgens (kR)}}$$

The ratio of factor mutations to biological damage means desirable changes free from associated undesirable changes on mutagenesis. Mutagenic efficiency also represents the proportion of mutations in relation

to biological damage. Mutagenic efficiency was calculated by the formula suggested by [6].

Mutagenic Efficiency =

$$\frac{\text{Mutation rate on the basis of M1 plant progenies (MP) or M3 population (MS)}}{\% \text{ pollen sterility or } \% \text{ reduction in plant survival}}$$

3. RESULTS AND DISCUSSION

Any mutational event may bring large or small change in the phenotype. The mutations affecting grass 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. A number of new commercial varieties have been originated from induced macro mutants and they proved their usefulness in attaining distinct breeding objectives. 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 characterized and named on the basis of specific characters constantly observed in them throughout the course of investigation. Effect of mutagens on the frequency and spectrum of different types of viable mutations in M₃ generation is illustrated in Table 1. The high frequency of mean mutations in different treatments was found under 40kR dry dose (4.280%) followed by 15kR dry dose (3.582%), 30kR dry dose (2.430%) and 35kR dry dose (2.275%), while the minimum (0.351%) was under dose 20kR pre-soaked treatment. It was clear that physical mutagen induced different mutation spectrum and types of mutant dependents not only on the types of mutagen but also on variety used as recorded in earlier studies [7] in chickpea, [8] in soybean, [9] in mustard.

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 [10] in bengal gram. This could be due to differential mode of action of the mutagens on different base sequences in various 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.

3.1 Types of Viable Mutations

3.1.1 Plant stature

3.1.1.1 Tall mutants

The tall mutants were isolated with a member of mutagenic treatments viz. 40kR (0.957) dry and 15kR (0.651) dry. These mutants were tall by at least 4-6 cm and high yielder than respective control. Same mutants have been reported by [11] in lentil, [12] in chickpea, [13] in chickpea. The number of primary branches, vigours growth and number of pods with bold seeds was increased.

3.1.1.2 Dwarf mutants

The dwarf mutants were isolated from many gamma treatments i.e., 25kR (0.741) dry and 30kR (0.405) dry. These are height characterized by condensed nodes, reduced height and lower seed yield as compared to respective controls. Similar dwarf mutants have been reported by [14,15,16] in green gram.

3.1.1.3 Semi dwarf mutants

Semi dwarf mutants were isolated from many gamma treatments viz., 40kR (0.478) dry and 10kR (0.347) dry. These mutants having normal height and these mutants matured about 6-8 days earlier than control. Semi dwarf mutants have been reported by [17] in urdbean.

3.2 Maturity Mutants

3.2.1 Early maturing mutants

These mutants were isolated at 20kR dry of gamma treatments. These mutants matured about 10-12 day earlier than control. Yield of these mutants were also found higher than the respective control. The results are akin to the finding of [16] in green gram and [17] in urdbean.

3.2.2 Late maturing mutants

The frequency of late maturing mutants were low observed at 05kR dry seeds. These late mutants measured about 4-6 day later then control. And yield of these mutants was found lower than respective control. Similarly late maturing mutant have been reported by [18] in Cowpea, [19] in Urdbean.

3.3 Pod Mutants

3.3.1 Bold pod mutants

These mutants were found in several gamma treatments viz., 15kR dry dose. These mutants had increased pod length, higher number of seeds per pod at higher seed yield as compared to the control.

3.3.2 Long pod mutants

The plants were normal in appearance with longer pods, containing 9 to 11 seeds per pod. Long pod mutants were recorded in gamma radiation treatment 20kR dry seeds.

3.3.3 Short pod mutants

Short pod mutants had relatively smaller pods and lower seed size as compared to the control. These mutants matured later than respective control.

Pod mutations such as bold, long and small were recorded in M₃ generation. Bold, long pod mutants were reported [20] in mungbean; [17] in black gram and small pod mutants, [21,22] isolated in chickpea and blackgram respectively.

3.4 Seed Colour Mutants

3.4.1 Brown seeded mutants

These mutants exhibited characteristics brown seed coat as compared to light green and white colour of the control seeds. These mutants had more number of pods per plant and more seed yield as compared to the control.

3.4.2 Light white mutants

These mutants exhibited characteristics light white seed coat colour as compare to light green colour of the control, however 100 seed weight of these mutant as less than control.

Seed colour mutants in brown (30kR) dry, light white (15kR) dry and light green (40kR) dry seeded mutants exhibited a characteristic pea seeds coat. Brown seed mutants were reported earlier by [23] and [17] in balckgram.

3.5 Seed Shape Mutants

3.5.1 Small seeded mutants

The mutants had relatively smaller and higher seeds as compared to the control and higher seeds as compared to the control and were low

yielder. Small seeded mutants were induced by 15kR (0.651) dry.

3.5.2 Bold seeded mutants

These mutants were induced by several gamma treatments. The seeds at these mutants were larger and heavier in comparison to the control and also exhibited increased 100-seed weight and seed yield as compared to the control.

3.5.3 Wrinkled seeded mutants

These mutants have wrinkled seed coat as compared to the round seed coat of respective control. These mutants were also having less seed yield and 100- seed weight as compared to control. These mutants of contributing characters viz., small, bold and wrinkled seeded mutant were isolated in M₃ generation. Such type of mutants was recorded earlier in [21] in chickpea.

In the macro mutants play an important role in plant breeding as it may lead to the evaluation of new genotypes. Significant morphological variability was created among mutants of pea using the gamma treatments. It was clear that physical mutagen induced different mutation spectrum and types of mutant dependents not only on the types of mutagen but also on variety used as recorded in earlier studies [6,9]. The more frequent induction of positive mutation types by exacting mutagen may be accredited to the information that the gene controlling these traits may be responsive to ionizing radiations. The viable mutants recorded in the present study included mutants with agronomically desirable features which could possibly be utilized in future breeding programme.

3.6 Mutagenic Effectiveness and Efficiency

Effectiveness means the rate of mutation induction as dependent upon the mutagenic doses and efficiency refers to the mutation rate in mutation to the various biological effects usually a measure of damage [24]. In M₃ generation of pea, mutagenic effectiveness was calculated on the basis of number of mutations per doses of mutagen. Mutagenic effectiveness was found to be higher at lower doses of gamma rays. It is including from the Table 2, showed that maximum effectiveness were found at 20kR dry (0.216) followed by 05kR pre-soaked (0.198) and 15kR dry (0.195), while lowest was found at the treatment of 25kR (0.089) dry. Similar results were recorded by [25] in black gram, [26] in Soybean and [27] in pea.

Table 1. Spectrum of macro mutations in M₃ generation of pea (cv. Arkel) under different levels of gamma treatments in rabi (2013-14)

S. no.	Mutation Types/Treatments	Plant stature			Maturity		Pod shape			Seed colour		Seed shape			Total frequency (%)	
		Tall	Dwarf	Semi Dwarf	Early	Late	Bold	Long	Short	Brown	Light white	Light green	Small	Bold		Wrinkled
1	Control (dry)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	05kR "	-	0.338	-	-	0.338	-	0.338	-	-	-	-	-	-	-	1.014
3	10kR "	-	-	0.347	0.347	-	-	0.347	-	-	0.347	-	-	0.347	-	1.735
4	15kR "	0.651	-	-	0.326	-	0.651	0.326	-	-	0.651	-	0.651	0.326	-	3.582
5	20kR "	0.360	-	-	0.719	-	-	0.719	-	-	-	-	-	0.360	-	2.158
6	25kR "	-	0.741	-	-	0.370	-	-	-	-	-	-	0.370	-	-	1.481
7	30kR "	-	0.405	-	-	0.810	0.405	-	0.405	0.405	-	-	-	-	-	2.430
8	35kR "	0.455	-	-	0.455	-	-	-	0.455	-	-	-	0.455	-	0.455	2.275
9	40kR "	0.957	-	0.478	0.478	0.478	0.478	-	-	-	0.478	0.478	-	-	0.455	4.280
10	Wet control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	05kR(Pre-soaked)	0.330	-	-	-	-	-	-	-	-	-	-	-	0.330	-	0.660
12	10kR "	-	-	-	-	-	-	0.353	-	-	-	-	-	-	-	0.353
13	15kR "	-	-	-	-	-	-	-	0.337	-	0.337	-	-	-	-	0.674
14	20kR "	-	-	-	-	-	-	-	-	-	-	-	0.351	-	-	0.351
Total		2.425	1.484	0.825	2.325	1.996	1.534	2.083	0.860	0.405	1.813	0.478	1.827	1.016	1.257	20.99

Table 2. Mutagenic effectiveness and efficiency of gamma rays in M₃ generation of pea cv. Arkel (2013-14)

S. no.	Mutation/Treatments	M ₃ plants (Numbers)	No. of mutated family	Pollen sterility per cent (PS)	Lethality or Plant survival per cent	Mutation rate(%)		Effectiveness		Efficiency	
						M ₃ plants (MS)	M ₃ plants (MS/dose)	MS/PS per cent	MS/ Plant survival per cent		
1	Control (dry)	-	-	-	-	-	-	-	-	-	-
2	05kR (dry)	296	2	10.06	13.78	0.676	0.135	0.013	0.049		
3	10kR "	288	4	11.38	12.04	1.389	0.139	0.012	0.115		
4	15kR "	307	9	10.16	10.46	2.932	0.195	0.289	0.280		
5	20kR "	278	12	10.12	11.56	4.317	0.216	0.427	0.373		
6	25kR "	270	6	22.02	18.73	2.222	0.089	0.101	0.119		
7	30kR "	247	7	32.62	46.59	2.834	0.094	0.087	0.061		
8	35kR "	220	9	48.29	71.29	4.090	0.117	0.085	0.057		
9	40kR "	209	14	53.12	85.77	6.699	0.167	0.126	0.078		
10	Control (wet)	-	-	-	-	-	-	-	-		
11	05kR(Pre-soaked)	303	3	13.45	11.27	0.990	0.198	0.074	0.088		
12	10kR "	283	5	15.18	21.21	1.767	0.177	0.116	0.083		
13	15kR "	297	7	18.85	40.12	2.357	0.157	0.125	0.059		
14	20kR "	285	8	19.57	53.70	2.807	0.140	0.143	0.052		

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 [6]. 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, most efficiency was exhibit 20kR dry (0.427 and 0.373) whereas minimum efficiency of mutation was found at treatment of 05kR (0.013 and 0.049) dry seeds. Similar results have been reported by [28] in chickpea; [29] in chickpea; [30] in *Vicia feba* (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.

4. CONCLUSION

It has been concluded from the analysis of the mutagenic effect for macro mutation, effectiveness and efficiency in subsequent generation of the present study that doses of gamma rays have great potential for heritable mutations in pea cultivar Arkel. Therefore, selected based on desirable agro-morphological traits of phenotypic M₃ putative mutants from different irradiative treatments of gamma rays, which showed stable phenotypes with complete penetrance and small variations in expressivity, could be advanced to next generations for yield, nutrition and adoptability assessment to release an extremely desirable pea mutant variety. 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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