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Research Article

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Phenotyping of Gamma Rays Induced Mutations in Lathyrus

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Abstract

An experiment on induction of mutation in lathyrus by gamma rays was conducted by using treated seeds of lathyrus cv. NLK-73 with 150, 200, 250, 300 and 350 Gy doses of gamma rays in non-replicated trial along with control. The treated material along with untreated control were sown in M_1 generation and the seeds collected from individual plant of M_1 generation were used to raise M_2 generation during *Rabi* 2017 and 2018 respectively. Fifteen different types of morphological and biochemical mutants *viz*., chlorophyll, early flowering, tall, broad leaves, profuse branches, more pods, flower colour, purple colour leaf, narrow leaves, more pods and tall, broad leaves and tall, broad leaves and profuse branches, profuse branches and tall, profuse branches and more pods, high yield and more pods were identified and isolated. Mutation frequency and mutagenic efficiency increased with the increase in dose upto 250 Gy and 200 Gy respectively, later on both decreased with increase in the dose of gamma rays. Mutagenic effectiveness decreased with the increase in doses of gamma rays. Significant variations among the treatments for all the mutants were recorded in M_2 generation of Lathyrus and hence, offer scope for identifying mutants.

1. Introduction

The *Lathyrus sativus* L. (2n=14) is an annual herb and one of the pulse crop rich in protein content (28%) next to soybean locally called as grass pea, khesari dal, peavine or chanamatra. It belongs to family Leguminoceae, sub family Papilionoideae and genus Lathyrus with 130 species occurring all over temperate region of Northern hemisphere and the higher altitude of tropical Africa. In India, besides the ornamental *Lathyrus odoratus*, the only other species cultivated is *Lathyrus sativus* which yield the khesari dal. Being a legume, it fixes atmospheric nitrogen through root nodules, part of which could be available to succeeding crop. It is mostly sown in standing crop of paddy as an 'Utera' or 'Paira' crop in *Rabi* season.

The lathyrus plant type is considered to be strongly drought resistant (Tripathy *et al*., 2011) and grows luxuriantly without any cultivation input. Beside this, Lathyrus contains toxic alkaloid which causes paralysis of lower limbs known as Lathyrism. The continuous consumptions of this pulse that too in undercooked condition of low temperature cause paralysis. In Maharashtra and some other states consumption, sale and cultivation of lathyrus was banned in 1961 because excessive consumption of lathyrus over a prolonged period during famine was reported to cause neurolathyrism a crippling disorder (Dwivedi and Prasad, 1964). Neurolathyrism is known to be caused by the neurotoxin, β-N oxalyl α,β-diamino propionic acid (ODAP) previously known as β-N oxalylamino L-alanine (BOAA).

Though the various improved varieties of lathyrus are developed by research scientists but area and average productivity of India is quite low in comparison to other countries. Grass pea is important pulse crop due to high protein (28-32%), next to soybean (42%). Therefore, to meet the protein need of large population of country different breeding strategies are being used in this crop to increase economic yield. Mutation is a sudden heritable change in the characteristics of an organism other than those due to mendelian segregation and recombination. Mutations are the rare events mostly recessive to wild type, often associated with deleterious or lethal effects and are inducible by a variety of external agents called mutagen. Spontaneous mutations occur naturally but are of rare occurrence and their frequency is also very low. Therefore, they don't solve the problems of breeder. The different genetical and breeding problems can be solved by inducing the artificial mutations in the organism and plants. Therefore, the technique of mutation breeding has been adopted as a valuable supplement to conventional breeding to create additional genetic variability. The variability created through these mutations is utilized for further crop improvement.

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The induced mutations technology for the crop improvement have successfully been used to create about 3222 mutant varieties around the world, of which India contributed 330 mutant varieties (Anonymous, 2017). Gamma rays mostly cause chromosomal and gene mutations. Analysis on effectiveness and efficiency of mutagen is an imperative tool in mutation breeding for crop improvement. The prior information of comparative effectiveness and efficiency of various mutagens facilitate the selection, which is essential to recover high frequency of desirable mutations (Smith, 1972; Kumar and Mani, 1997). Although, effectiveness and efficiency of a particular mutagen are completely different properties but they together define the usefulness of any mutagen. It is not necessary that an effective mutagen shall be an efficient one also (Gaikwad and Kothekar, 2004). The value of effectiveness and efficiency estimation depends on various factors like biological, environmental and chemical that can modify mutation rate of different mutagens (Kodym and Afza, 2003).

The objectives of the present study were to assess the comparative effectiveness and efficiency of on the induction of genetic variability for identifying the optimum concentrations of mutagens for induced mutagenesis in *Lathyrus sativus* (L.) and to assess the comparative sensitivity of the different phenotypic categories of Lathyrus for understanding the spectrum of mutations induced by the different concentrations of mutagens.

2. Materials and Methods

In the present investigation dry, healthy and genetically pure seeds of *Lathyrus sativus* cv. NLK-73 (high yielding cultivar) were obtained from Agril. Botany section, College of Agriculture, Nagpur. Six different lots of 500 seeds of Lathyrus seed cultivar NLK-73 were made. Out of these five lots of seeds were sent to Bhabha Atomic Research Centre, Trombay, for irradiation with five different doses of gamma rays *i.e.*, 150 Gy, 200 Gy, 250 Gy, 300 Gy and 350 Gy (Co60 at BARC, Trombay, Mumbai) and used for raising M_1 during *Rabi* 2017 and individual plant in each treatment were harvested separately. The harvested seed were used to raise M_2 generation in *Rabi* 2018 and mutants were identified.

M_2 generation was raised in *Rabi* 2018. Fifteen hundred seeds of each treatment from M_1 generation were sown to raise M_2 population. The sowing was undertaken on the fertile and well leveled piece of land in the field of Agril. Botany section, College of Agriculture, Nagpur. The M_2 population was observed for different parameters besides scoring of different mutants. The treated populations were carefully screened for all morphological characters. The spectrums of mutations were scored treatment wise to study the mutagenic effectiveness and efficiency of each treatment. The mutation frequency in percentage for induced visible mutants was calculated for each treatment as suggested by Gaul (1958) and the efficiency and effectiveness of mutagen in different treatments in M_2 generation were estimated as per the formula given by Konzak *et al.* (1965).

$$\text{Mutation frequency (\%)} = \frac{\text{Number of visible mutants scored}}{\text{Total population in a treatment}} \times 100$$

$$\text{Mutation efficiency} = \frac{\text{Mutants per 100 } M^2 \text{ plants}}{\text{Number of visible mutants scored}}$$

$$\text{Mutation effectiveness} = \frac{\text{Mutants per 100 } M^2 \text{ plants}}{\text{Dose of radiation in gamma rays}}$$

3. Results and Discussion

In the present investigation 15 different types of morphological and economical mutants were identified and isolated from M_2 population as presented in table 1.

3.1 Tall Mutant

The tall mutants were induced in all the treatments. The maximum frequencies were obtained in 300 Gy (1.64%) and the low frequencies in 200 Gy (1%). The range for the character was from 85 to 118 cm as compared to control (58 cm). In accordance to this result Gobinath and Pavadai (2015) in soybean also obtained tall mutant and recorded moderate and high mean value for plant height in 50 Kr of gamma rays and 0.5% EMS as compared to control.

3.2 Purple Pigment Leaf Mutant

The purple pigment leaf mutants were isolated from all the treatments. The highest frequency for the character was observed in 300 Gy (1.64%) followed by 350 Gy (1.54%), 250 Gy (1.39%), 200 Gy (1.16%) and the lowest in 150 Gy (1.12%).

3.3 Broad Leaves Mutant

Broad leaf mutants were found in all the treatments. The highest frequency was found in 350 Gy (1.54%) followed by 150 Gy (1.44%), 200 Gy (1.33%), 300 Gy (1.28%) and 250 Gy (1.04%). These mutants had broad leaves as compared to control. The broad leaves mutants were also earlier reported by Laskar and Khan (2017) in lentil at frequency of 0.17% in cv. DPL-62 and 0.19% in cv. Pant L-406 by gamma rays treatment.

3.4 Narrow Leaves Mutant

Narrow leaves mutant were found in all the treatments. The highest frequency was observed in 350 Gy (1.16%) followed by 200 Gy (0.50%), 150 Gy (0.48%), 300 Gy (0.36%) and 250 Gy (0.35%). These mutants had narrow leaves as compared to control. The narrow leaves mutants were earlier reported by Laskar and Khan (2017) in lentil at frequency of 0.13% in cv. DPL-62 and 0.19% in cv. Pant L-406 of the gamma rays treatment.

3.5 More Number of Pods Mutants

The more number of pods mutants were isolated from all the treatments. The highest frequency for the character was observed in 250 Gy (2.26%) and the lowest in 300 Gy (0.36%).

Mutants recorded a range of 232 to 469 pods as compared to their respective control (117 pods). Similar to the above result Rybinski *et al.* (2006) reported that in grass pea the number of pods plant^{-1} was increased. The number of pods plant^{-1} ranged from 37.1 to 95.4 as compared to untreated cv. Krab (56.2 to 69.8).

3.6 More Branches Mutant

The more branches mutants were isolated from all the treatments. The highest frequency for the character was observed in 300 Gy (1.28%) and the lowest in 200 Gy (0.66%). Mutants showed increased height and more number of branches (6 to 9 branches) as compared to their respective control (5 branches). The more branches mutants were earlier reported by Yaqoob and Ahmed (2003) in mungbean, gamma rays were found to be more effective than EMS for more branched mutants.

3.7 Flower Colour Mutant

The flower colour mutants (Figure 1) were isolated from almost all the treatments except 250 Gy. The highest frequency for the character was observed in 300 Gy (0.36%) and the lowest in 200 Gy (0.17%). Mutants showed pink colour flower (150, 200, 300 and 350 Gy) and white flower colour (300 Gy) against blue in control. Similar to these results Ramezani *et al.* (2016) in grass pea reported different flower colour mutants like white, purple, pink and yellow in gamma rays treatments as against blue in control.

Figure 1: Flower colour mutants

3.8 Chlorophyll Mutant

The chlorophyll mutants were isolated from all the treatments. The highest frequency for the character was observed in 300 Gy (1.82%) followed by 350 Gy (1.73%), 250 Gy (1.56%), 150 Gy (1.28%) and the lowest in 200 Gy (1.16%). In accordance to these results Ramezani and More (2014) in grass pea reported a progressive increase in mutation frequency of chlorophyll mutants with increasing doses. Three different types of chlorophyll mutants, such as, albino, xantha and viridis were induced due to the effect of mutagens. Khan and Tyagi (2013) also isolated four types of mutants *viz*., albino, xantha, chlorine and viridis in two cultivars of soybean (Pusa-16 and PK-1042).

3.9 Early Flowering Mutant

Early flowering mutants were isolated from the 150, 200 and 300 Gy treatments. The highest frequency for the early flowering mutant was observed in 300 Gy (0.36%) followed by 150 Gy (0.32%) and the lowest in 200 Gy (0.17%). These plants flowered in 10 to 12 days (48 to 50 days) earlier than their control (60 to 62 days). Tulmann and Alves (1997) observed early flowering mutant in soybean cv. Parana treated with 25 kR gamma radiation.

3.10 More Pods and Tall Mutant

More pods with tallness mutants were isolated from all the treatments. The highest frequency for more pods with tallness mutant was observed in 200 Gy (1.0%) followed by 150 Gy (0.96%), 300 Gy (0.55%), 350 Gy (0.19%) and the lowest in 250 Gy (0.17%). The more pods with tallness mutant were earlier reported by Baisakh *et al.* (2014) in Blackgram.

3.11 Broad Leaves and Tall Mutant

Broad leaves with tallness mutants were isolated from the 150 and 300 Gy treatments. The highest frequency for the broad leaves with tallness mutant was observed in 300 Gy (0.18%) and the lowest in 150 Gy (0.16%). Mutants showed broad leaves with increased plant height (85 to 136 cm) as compared to their respective control (35 to 85 cm).

3.12 Broad Leaves and More Pods Mutants

Broad leaves with more pods mutants were isolated from the

200 and 350 Gy treatments. The highest frequency for the broad leaves with more pods mutant was observed in 200 Gy (0.33%) and the lowest in 350 Gy (0.19%). Mutants showed broad leaves with increased number of pods (232 to 469 pods) as compared to their respective control (85 to 177 pods).

3.13 More Branches and Tall Mutant

The more branches mutants with tallness were isolated from the 150 Gy and 250 Gy treatments. The highest frequency for the character was observed in 250 Gy (0.35%) and the lowest in 150 Gy (0.16%). Mutants showed increased height and more number of branches (6 to 9 branches) as compared to their respective control (3 to 5 branches).

3.14 More Branches and More Pods Mutant

The more branches with more number of pods mutant were isolated from the 200 Gy and 350 Gy treatments. The highest frequency for the character was observed in 200 Gy (0.33%) and the lowest in 350 Gy (0.19%). Mutants showed increased in number of branches (6 to 9 branches) and more number of pods (232 to 469 pods) as compared to their respective control (3 to 5 branches and 85 to 177 pods).

3.15 High Yield and More Pods Mutant

The more pods with high yield mutant (Figure 2) were isolated from almost all the treatments. The highest frequency for the character was observed in 250 Gy (8.16%) and the lowest in 350 Gy (0.39%). Mutants showed increased range for number of pods (232 to 469 pods) and high yield (41.9 g to 92.0 g) as compared to their respective control 177 pods and 30.2 g yield. Rybnski *et al.* (2004) reported that the traits which have a significant effect on the yield of seeds is the number of pods plant^{-1} in mutants of lathyrus.

Figure 2: Mutants for high yield and more pods

3.16 Mutation Frequency in M_2 Generation

Mutation frequency of each visible mutant in M_2 generation was calculated and presented in table 1 and graphically in figure 1. The table revealed that the treatment 250 Gy gamma rays induced the highest mutation frequency (15.10%) followed by 200 Gy (13.79%), 150 Gy (11.64%), 300 Gy (11.11%) and the lowest in 350 Gy (10.40%). The frequency of mutation was comparable in all the treatments. The mutation frequency increased with the increase in dose up to 250 Gy after that decreased with the increase in dose of gamma rays. Similar to these results Girija and Dhanavel (2009) reported that the mutation frequency decreased with the increase in dose or concentration of mutagen in cowpea. The minimum chlorophyll and viable mutation frequency was observed in 30 kR of gamma rays. Tripathy *et al.* (2011) also reported that frequency of mutations increased with the doses of gamma rays almost in geometric progression upto 40 kR and thereafter increased with decreasing rate.

3.17 Mutagenic Efficiency and Effectiveness

The efficiency and effectiveness of mutagens were estimated and are presented in table 2. It is noticed that 200 Gy exhibited the highest mutagenic efficiency (0.32), while 350 Gy (0.19) showed the lowest. It was observed that the mutagenic efficiency increased with the increase in doses up to 200 Gy and after that decreased with the increase in doses of gamma rays. Among the treatments the highest mutagenic effectiveness was observed in 150 Gy (0.077) followed by 200 Gy (0.068), 250 Gy (0.060) while the lowest was noticed in 300 Gy (0.037) and 350 Gy (0.030). Further it was noticed that the mutagenic effectiveness decreased with the increase in the dose of gamma rays. In accordance to the above result Girija and Dhanavel (2009) also reported increase in mutagenic

Table 1: Frequency of induced mutants in different gamma rays treatments in M_2 generation

Sl. No.	Mutants	T_1 (150 Gy)	T_2 (200 Gy)	T_3 (250 Gy)	T_4 (300 Gy)	T_5 (350 Gy)	Total
1	Tall plant	1.44	1.00	1.22	1.64	1.35	6.64
2	Purple pigment leaf	1.12	1.16	1.39	1.64	1.54	6.85
3	Broad leaves	1.44	1.33	1.04	1.28	1.54	6.62
4	Narrow leaves	0.48	0.50	0.35	0.36	1.16	2.84
5	More pods	0.64	1.33	2.26	0.36	1.35	5.94
6	More branches	0.96	0.66	1.04	1.28	0.77	4.71
7	Flower colour mutant	0.32	0.17	-	0.36	0.19	1.04
8	Chlorophyll mutant	1.28	1.16	1.56	1.82	1.73	7.56
9	Early flowering	0.32	0.17	-	0.36	-	0.85
10	More pods and tall	0.96	1.00	0.17	0.55	0.19	2.87
11	Broad leaves and tall	0.16	-	-	0.18	-	0.34
12	Broad leaves and more pods	-	0.33	-	-	0.19	0.52
13	More branches and tall	0.16	-	0.35	-	-	0.51
14	More branches and more pods	-	0.33	-	-	0.19	0.52
15	High yield and more pods	2.39	1.65	5.73	1.28	0.19	14.24
	Total	11.64	13.79	15.10	11.11	10.40	62.05

efficiency and effectiveness with the decrease in dose in cowpea. Khan and Tyagi (2013) in soybean reported that the mutagenic effectiveness and efficiency decreased with the increase in dose of mutagen in both the cultivar Pusa-16 and PK-1042. Among the different treatments studied T_3 (250 Gy) was found to be more effective as this treatment gave maximum number of mutants followed by T_2 (200 Gy).

Table 2: Effect of gamma rays treatments on mutation frequency and mutagenic efficiency and effectiveness in M2 generation

Treatments	Percent lethality	Mutation frequency (%)	Mutagenic efficiency	Mutagenic effectiveness
T_1 (150 Gy)	41.05	11.64	0.28	0.077
T_2 (200 Gy)	42.94	13.79	0.32	0.068
T_3 (250 Gy)	47.81	15.10	0.31	0.060
T_4 (300 Gy)	50.50	11.11	0.22	0.037
T_5 (350 Gy)	53.00	10.40	0.19	0.030

4. Conclusion

It is concluded from this study that total 15 different types of morphological mutants were obtained from which 123 desirable and superior single plant mutants and were identified for testing in M_3 generation. It was observed that the number of desirable mutants identified were maximum of 47 in T_3 (250 Gy) followed by T_2 (200 Gy) in which 43 mutants were selected. The mutants identified in other treatments were 23 in T_1 (150 Gy), 8 in T_4 (300 Gy), 2 in T_5 (350 Gy). All these mutant plants were suggested to be forwarded to M_3 generation as progeny rows till homozygosity is reached and after attainment of homozygosity superior progenies can be evaluated in yield trials.

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