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STUDY OF PHENOTYPIC VARIABILITY INDUCED BY PHYSICAL AND CHEMICAL MUTAGENS IN *LATHYRUS SATIVUS* (V. P-24)

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***Abstract*:** Physical and chemical mutagens have induced various types of mutations useful in the improvement of crops. In recent years it has been shown on various plant species that the effect of mutagen treatment varies with varying doses and varying periods of treatments. The paper reports the results of application of 5kr, 10kr and 15kr of gamma rays and 0.02% of Nitroso Methyl Urea (NMU) on Khesari (*Lathyrus sativus*) var. P-24. Saperate and combined doses of gamma rays with 0.02% NMU were used for mutagenic effects. Observations for M_2 generation were recorded at a regular interval. Mutants for seedling morphology like curled apex, stunted seedling, radicle hypertrophy, folded leaflets and mutants for plant height like dwarfs, stunted, branching habit mutants, leaf mutation and mutants for flowering were observed.

Index Terms: Gamma rays, NMU, induced mutation, grass pea.

Introduction: Grass pea is an important crop of economic significance in India, Bangladesh, Pakistan, Nepal, and Ethiopia. In India it is extensively cultivated in Madhya Pradesh, Bihar and some extent in Uttar Pradesh; Bengal, Andhra Pradesh and Maharashtra. It is known locally as *Khesari, Teora, Metra, lakh, lankalu* etc. the grass pea is endowed with many properties that combine to make it an attractive food crop in drought-stricken, rain-fed areas where soil quality is poor and extreme environmental conditions prevail (Palmer *et al.*, 1994). Indian population is mainly dependent upon the pulses for fulfilling their protein requirement because of high cost of animal based proteins and their vegetarian food habits (Yadav, 1991). Mutation breeding combines several advantages in plant improvement by upgrading a specific character without altering the original genetic make-up of the cultivar and is a well functioning branch of plant breeding, supplementing to conventional methods in a favourable manner (Toker *et al.*, 2007). Simultaneous treatment of different mutagens could be used to increase mutation frequency (Wani, 2009). Physical and chemical mutagens have induced various types of mutations useful in the improvement of crops. In recent years it has been shown on various plant species that the effect of mutagen treatment varies with varying doses and varying periods of treatments. Mutation research on grass pea has gained momentum thereafter bringing about arrays of mutagenic changes involving chromosomal anomalies, chlorophyll deficiencies and different types of phenotypic modifications (Prasad and Das, 1980; Waghmare *et al.*, 2001; Talukdar *et al.*, 2006; Rybinski, 2003). The morphological mutants are useful as novel genetic variants to elucidate genetic basis of desirable traits in grass pea genetics and breeding. The present study reports the effects of gamma rays as a physical mutagen and Nitroso Methyl Urea as a chemical mutagen. The observations were based on phenotypic traits in M_2 generation of grass pea.

Material and Methods: Dry, dormant and healthy seeds of grass pea (*Lathyrus sativus)* var. P- 24 were subjected to CO^{60} gamma irradiation at the Nuclear Research Lab, I.A.R.I. New Delhi. Three different doses of gamma rays 5kr, 10kr and 15kr were applied. A part of seeds from each irradiation treatment and a sample of fresh untreated seeds were soaked in 0.02% NMU (Nitroso methyl Urea) for six hours. After soaked in NMU the seeds were thoroughly washed in running tap water for 8 to 10 times. A sample of untreated seeds was used as control. Thus there were eight treatment combinations including the control. The treated seeds were sown in the field along with the control in a randomized block design. Observations for M_2 generation were recorded at a regular interval. Mutants for seedling morphology like curled apex, stunted seedling, radicle hypertrophy, folded leaflets and mutants for plant height like dwarfs, stunted, branching habit mutants, leaf mutation and mutants for flowering were observed.

Results and Discussion:

All the plants in M_1 generation were harvested separately treatment wise. Seeds from individual M_1 were sown to raise the M_2 population in a plant to family manner. Important viable mutants involving plant height, leaf shape and size, flowering time, multiflorate and multicolored characters, pigmentation of aerial parts and nature of tendrils were recorded. The frequencies of vital mutations were observed in M_2 generation. Among the three doses of gamma rays, the frequency of mutations in terms of percentage of families segregating as well as in terms of mutants per thousand M_2 plants, increases with an increase in the dose of radiation. NMU induced a higher frequency of segregating families than the 5 KR or 10 KR doses of gamma rays, but it was lower than that induced by the 15 KR doses of gamma rays. However, the proportion of mutants per thousand M_2 plants in the NMU treatment was much higher than any of the gamma ray treatment. In the combined treatments of two mutagens the frequency of mutations increased with the increase in dose of radiation. It was maintained both in terms of segregating families and in terms of per thousand M_2 plants. In each of the three combined treatments, mutants per thousand M_2 plants were higher than the respective single application of gamma rays or NMU.

Mutants for seedling morphology: Besides the chlorophyll mutations certain seedlings with morphological anomalies such as curled apex, stunted growth, radicle hypertrophy, barren apex, folded leaflets and giant seedlings were recorded under all the treatments. The spectrum of these mutants under various treatments is also presented in table 1. Characteristics of various seedling morphology mutants are described here briefly.

1. Curled apex: Seedlings with curled apex were normal up to the level of cotyledonary leaves. But above the cotyledonary leaves the shoot apex turned downwards and gave a twisted appearance. The curled apex mutants failed to survive and dried up twenty to twenty five days after their emergence. These mutants were more frequently induced in the gamma ray treatments or the combined treatments of the two mutagens while their frequency in the NMU treatment was very low.

2. Stunted seedlings: Characteristics of the stunted seedlings were reduced height and shorter internodes length as compared to the normal seedlings. Most of these mutants survived up to maturity when transferred to the field. These mutants also were more frequently induced by gamma rays or the combined treatments than by NMU. Combined treatments induced a lower frequency of stunted seedlings than the respective gamma ray treatments.

3. Radicle hypertrophy: Mutant seedlings of these categories did not grow beyond the cotyledonary leaf stage. When these seedlings were dug out of the sand, it was observed that the base of the radicle was hypertrophied due to formation of a ball like callus tissue. The thin radicle tip extending beyond the callus soon dried up, which ultimately caused the death of the seedlings. These mutants were induced by gamma rays alone or in combined treatments with NMU, while NMU alone failed to induce radicle hypertrophy. Occurrence of these mutants in a much higher frequency in the lowest dose of gamma rays in comparison to the other treatments is inexplicable.

4. Barren apex and folded leaflets: Seedlings with barren apex developed normally up to three to four leaf stage. After this stage no leaves were developed on the shoot apex. Mutants of this kind, which failed to survive and dried up within three weeks of emergence, were induced by the action of gamma rays, either in individual applications or in combination with NMU. Anomaly folded leaflet was more common in mutants induced by the combined treatments of the two mutagens than by the individual application of either of the mutagens.

5. Giant seedlings: Seedlings of normal treatment attained a height of 4 cm to 6 cm after fifteen days of sowing. The giant seedlings, however, became as tall as ten to fifteen centimeter within the same period. The enormous increase in height was due to a very rapid elongation of the hypocotyle. Further growth of the giant seedlings was retarded and all of them dried up within twenty to twenty five days of sowing. Giant seedlings were more frequent in the NMU treatment and in the combined treatments with gamma rays.

Treatments	Spectrum Of Seedling Morphology Mutants							
	Curled Apex	Stunted	Radicle Hyper-Trophy	Bilobed Cot. Leaves	Tricotyle	Barren Apex	Folded Leaflets	Giant Seedlings
Control	-	-	-		-	-	-	-
Gamma Rays 5 KR	**4.50**	**9.87**	**12.05**	**2.20**	**1.95**	-	**6.08**	**1.10**
10 KR	**4.55**	**17.80**	-	-	**1.52**	-	-	-
15 KR	**12.02**	**23.00**	**2.25**	-	**1.24**	**2.27**	**2.27**	-
NMU 0.02%	**1.81**	**4.36**	-	**9.20**	**11.26**	-	**3.75**	**3.75**
Gamma Rays + NMU 5 K + 0.02%	**16.52**	**6.09**	**2.49**	-	**10.70**	**9.27**	**20.50**	**15.07**
10 KR + 0.02%	**11.12**	**7.07**	**3.19**	**4.00**	**7.09**	**8.01**	**8.91**	**4.21**
15 KR + 0.02%	**8.05**	**13.60**	**0.77**	**3.10**	**5.00**	-	**5.95**	**2.10**

Table 1. Mutation spectrum for seedling morphology in M_2 generation of mutagen treated grass pea**.**

Mutants for plant height: Average plant height in the control was 60 cm (Fig1). Various mutants for plant height observed in the M2 population were as follows:

1. **Semi-dwarfs:** Height 35 to 40 cm (Fig. 2). **2. Dwarf:** Height ranging from 12 to 15 cm (Fig. 3). **3. Bushy dwarfs:** Plant height equal to the dwarf mutants but the number of secondary and tertiary branches was much increased (Fig. 4). **4. Stunted:** Miniature mutants with height ranging 3.5 to 6 cm having curled leaves (Fig. 5).

All the four categories of plant height mutants were recorded in the M_2 progeny of all the seven treatments. However, the frequency of mutants related to the height was much higher in the individual applications of NMU or in combined application with gamma rays than individual applications of gamma rays.

Branching habit mutants: Mutants showing marked variations in the branching habit of the plant were induced in all the treatments. In the control plants there are four to six primary branches each of which gives out several secondary branches. Enhanced branching mutants had six to eight primary branches and profuse secondary branches whereas, sparse branching mutants had a single or two primary branches and number of secondary branches in them was also greatly reduced. Some mutants showed extra enlarged branches (Fig.6) while other mutants showed stunted branches (Fig. 7). These branching habit mutants were quite frequent in all the treatments.

Leaf mutations: Three different categories of mutations effecting dimensions of leaves and leaflets were recorded. Mutants with leaves showing an elongation of the rachis and increase in the length and width of the leaflets were termed as 'large leaf mutants' while mutants where length of the leaflets was normal or slightly increased but their width was very much reduced were termed as 'narrow leaflet mutants'. In some of the mutants both length and width of the leaflets was markedly reduced and only a few leathery leaflets or only rudimentary leaflets were arranged irregularly on the shortened rachis. These mutants were termed as 'small and narrow leaflet mutants'. All of these mutants were more frequent in combined treatments.

1. Plants with normal growth.

2. A semi-dwarf plant.

3. A dwarf plant.

4. A bushy dwarf plant.

5. A stunted plant with curled apex.

6. A plant with enlarged branches and internodes.

7. A plant with short branches.

Tendrillar mutants: Usual presence of long terminally coiled tendril in compound leaf of grass pea varieties was modified to short curly, straight, unbranched, abnormal branching, hooked and highly coiled tips. The tendril less mutant produced a tuft of small leaflets in place of terminal tendril in compound leaf.

Mutants for time taken to flowering: The normal time taken to flowering in the control was 90 days from the date of sowing. Variation in the number of days taken to flowering was observed in the M2 populations of different treatments. The mutants were classified as "very early flowering" (70 to 75 days), "early flowering" (75 to 85 days), "late flowering" (100 to 110 days) and "very late flowering" (111 to 120). Most of the mutants for maturity period were more frequently induced by combined treatments of both the mutagens than individual treatment of any of them. The frequency of maturity period mutants increased with the increase in radiation dose among the gamma ray treatments as well as the combined treatments.

Flower colour mutants: These mutants had white-purple colour flowers instead of purple colour of the control. These mutants were detected in some plants of 15KR and NMU treatment. The colour of the plant body was light green in comparison to dark green in the control. Height of these mutants was normal (fig. 8 and 9).

8. A normal plant with purple flowers.

9. A mutant plant with white-purple flowers.

Acknowledgement: Based on the results obtained in the present study, it can be concluded that the combined application of physical and chemical mutagens is much more effective than the use of individual mutagen to produce genetically new mutants.

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