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Short Communication

Genetic Variability Induced by Gamma Rays and NMU in Grass Pea (*Lathyrus sativus*) Var. P-24

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***Key words*:** *Lathyrus sativus*, gGamma rays, NMU, Meiosis, Chromosomal aberrations

Grass pea (*Lathyrus sativus*) is an important pulse crop being a better source of protein among the pulses. Historically, grass pea has been a daily food for millions in Asia and Africa. It may be used for cytogenetic studies and producing new offspring without neurotoxin. The genetic variability may be achieved through mutagenesis. Some of the induced mutations may be useful for cultivation caused by physical as well as chemical mutagens in plants. These mutagens are being used in genetic improvement program of different plant species. Simultaneous treatment of different mutagens could be used to increase mutation frequency [1]. The physical and chemical mutagens are known to produce chromosomal aberration [2]. Mutagenic changes in grass pea are involving chromosomal anomalies, chlorophyll deficiencies and different types of phenotypic modifications [3]. The study of pollen and ovule sterility is another criterion for determining mutagenic sensitivity [4]. Cytological abnormalities induced by the mutagens have been extensively studied by various workers in several crops. The present study reports the effects of gamma rays as a physical mutagen and Nitroso Methyl Urea (0.02%) as a chemical mutagen. Eight different seed treatments were used of gamma rays and NMU. The observations were based on chromosomal aberrations in pollen grains of grass pea.

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, Indian Agricultural Research Institute, 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. At the time of flowering fresh flowering buds from all the plants under different treatments were collected and fixed in 1:3 acetic acid and alcohol for 24 hours and transferred to 70% alcohol. Uniformly stained pollen grains with aceto orcein were taken to test their sterility and chromosomal changes. Slides of each treatment were observed under phase contrast photographic microscope and scored to cover maximum surface area of the slide.

Fig 1 Meiotic configuration in control

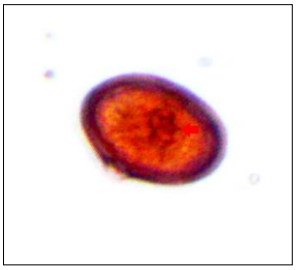

Fig 2 Stickiness at metaphase

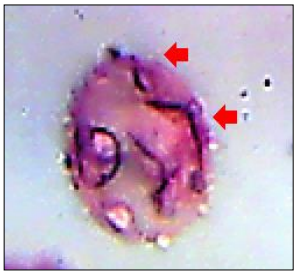

Fig 3 Dense and enlarged bivalents

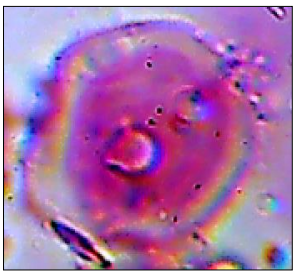

Fig 4 Ring formation at anaphase

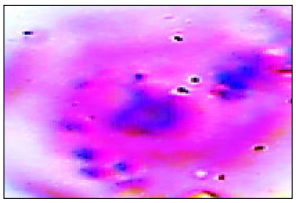

Fig 5 Laggards at anaphase

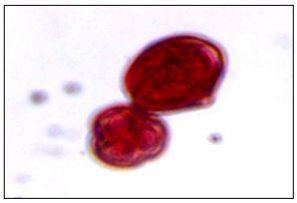

Fig 6 Cytoplasmic connection between PMCs

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. It provides a rapid method to improve local crop varieties, without going through extensive hybridization and back crossing used in

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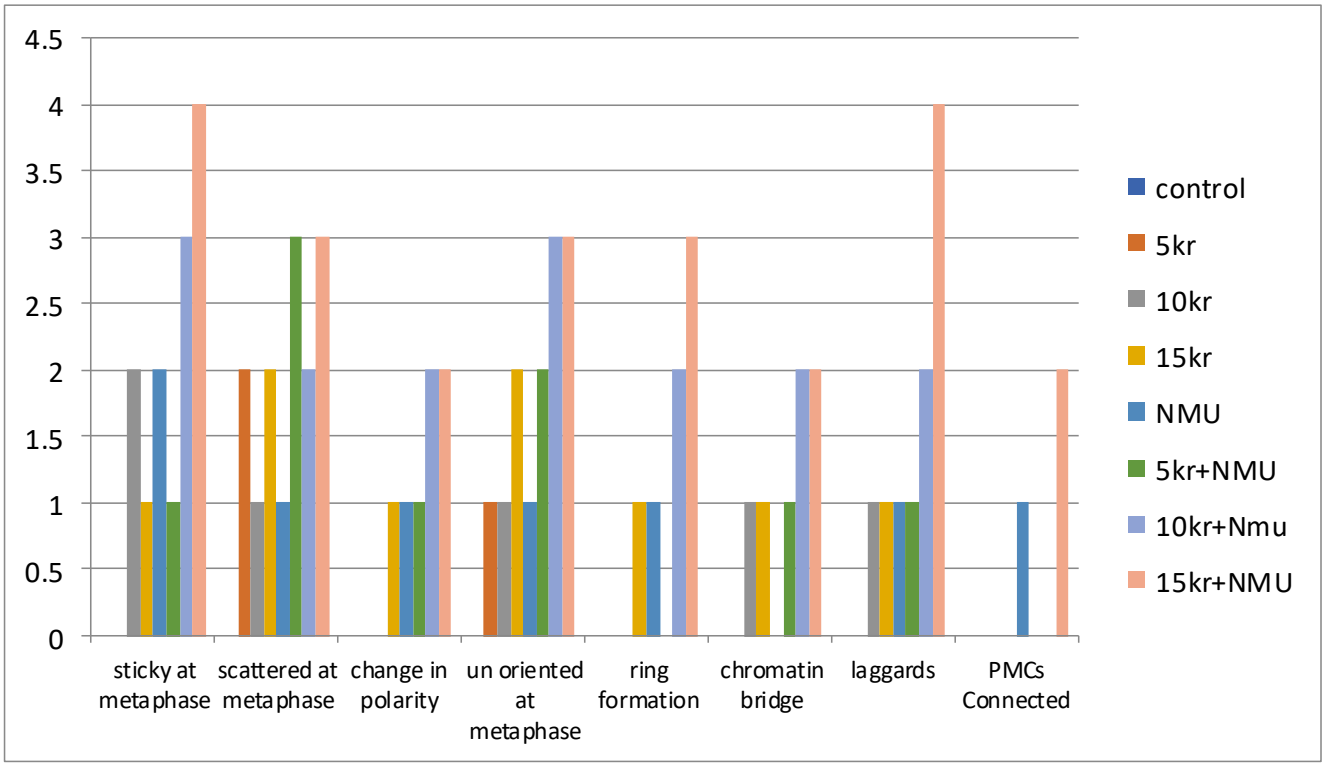
conventional breeding. Physical and chemical mutagens have induced various types of mutations useful in the improvement of crops. The properties and mode of action of physical and chemical mutagens has also been reviewed by [5], [6], [7] who enriched our knowledge on fundamental aspects of the mutational process and the possible mechanism of action of various physical and chemical mutagens. 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 present investigation has been carried out to mutagenic effects of gamma rays and NMU on the pollen grains of grass pea. It was observed that higher doses of mutagens induce maximum genetic variability. Conventional cytological analysis was conducted and the presence of 7 bivalents at metaphase- I and regular separation of 7:7 at anaphase- I was observed in the control (Fig 1). But several cytological anomalies were revealed in the pollen mother cells of mutagenic treated plants. Most of the abnormalities recorded in the metaphase I and anaphase I stages of treated plants. Stickiness (Fig 2) and enlarged bivalents (Fig 3) at metaphase I, ring formation (Fig 4) and laggards (Fig 5) at anaphase I was observed in the treated plants. Cytoplasmic connection between the pollen mother cells (Fig 6) was also observed. Maximum abnormalities were recorded in higher doses of gamma rays and 0.02% NMU (Table 1, Graph 1). The similar differences in mutagenic response have also been reported by many workers [8], [9]. Aberrations increased along with the increasing concentration of the mutagens have been reported by [10] in *Cichorium intybus* L. The varied degree of effectiveness and efficiency varied between different mutagens and also between varieties has been reported in the chickpea [11].

Table 1 Chromosomal aberrations observed during different stages of meiosis in pollen mother cells

Chromosomal alterations	Control	5 KR	10 KR	15 KR	0.02% NMU	5 KR + NMU	10 KR + NMU	15 KR + NMU
Sticky at metaphase	-	-	2	1	2	1	3	4
Scattered metaphase	-	2	1	2	1	3	2	3
Change in polarity	-	-	-	1	1	1	2	2
Un oriented metaphase	-	1	1	2	1	2	3	3
Ring formation at anaphase	-	-	-	1	1	-	2	3
Chromatin bridge	-	-	1	1	-	1	2	2
Laggard at anaphase	-	-	1	1	1	1	2	4
Cytoplasmic connection PMCs	-	-	-	-	1	-	-	2
Total	-	3	6	9	8	9	16	23



Graph 1 Showing frequency of chromosomal aberrations induced by mutagens

Combined treatments of different mutagens increase the mutation frequency and alter the mutation spectrum [12]. Maximum abnormal dividing cells have been reported in chickpea when combined treatment of EMS and gamma rays was applied by [13]. The combined treatments of physical and chemical mutagens induced a wider range of mutation spectrum, which is of great significance to the experimental mutagenesis [14]. Dose dependent increases in meiotic

abnormalities in mung bean with EMS and HZ (Hydrazine Hydrate) treatment [15]. Earlier dose dependent increase in meiotic abnormalities has also been reported in mung bean [16], in cereals [17]. Separate and combined implementation of physical and chemical mutagens showed significant effects on dividing cells [18]. The combined treatment of gamma rays and 0.02% nitroso methyl urea (NMU) showed more potent effects as compared to independent uses of both the mutagens. In the present study, percentage of pollen sterility increased with an increase in the doses of gamma rays. The maximum pollen sterility was observed in combined treatment of 15kr of gamma rays and NMU.

SUMMARY

Chromosomal variation is an important tool in genetic analysis. The objectives of the present study were to determine mutagenic efficiency of physical and chemical mutagens. The paper reports the results of application of 5kr, 10kr and 15kr of gamma rays and/ or 0.02% of Nitroso Methyl Urea (NMU) on Khesari (*Lathyrus sativus*). The seeds of *Lathyrus* were treated with 5kr, 10kr and 15kr doses of gamma rays and 0.02% of NMU independently and in combination. Different types of chromosomal changes were observed during meiotic division like stickiness, laggards, bridges and condensed bivalents etc. The higher doses of gamma rays showed deleterious effects when applied separately as well as combined with NMU. The combined treatment of physical and chemical mutagens was more effective than independent doses of both mutagens.

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