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Research Article

Spectrum and frequency of Chlorophyll mutation in grass pea (*Lathyrus sativus* L)

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Abstract

The seeds of grass pea (*Lathyrus sativus* L.) various chemical mutagen concentrations (EMS 0.05%, 0.10%, 0.15%; SA 0.005%, 0.010%, 0.015% and NMU 0.15%, 0.30%, 0.45%) and physical mutagen doses (Gamma rays- 15KR, 30KR, 45KR) were treated in laboratory. The Treated and untreated seeds were sown in the field by CRBD method. The growth of M1 generation observed the characters and number of mutants in each population thoroughly. The chlorophyll mutation frequency was calculated on the basis of plant population with explicit chlorophyll mutation and leaf phenotypic diversity. A noticeable varied range of chlorophyll and morphological mutants were observed in the M1 generations. Distinct kinds of spectrum of chlorophyll mutants' viz. xantha, viridis, albino and chlorina were identified in the treated population. Prominent phenotypic aberrations were also noted viz. reduction in size of leaves, obtuse and retuse apex, notch at margin, bilobed leaf, lanceolate leaf, wrinkled leaflet and triforked leaf, etc. The frequency of leaf morphological changes observed in almost all chemical and physical mutagen in grass pea.

INTRODUCTION

Grass pea (*Lathyrus sativus* L.) is an annual winter pulse crop in India occupying an area of about 1.6 million ha (FAO, 2002) and about nearly 200 species and subspecies. *L. sativus* is the only member used as a grain legume (Biswas, 2007). Cultivation was doing for more than 8000 years, a very less evolutionary progress and improvement of this crop was made as a pulse crop mainly due to narrow genetic variability and unavailability of marker genes with prominent morphological manifestations, suitable for comprehensive genetic studies and breeding for high yield of low seed neurotoxin (ODAP) lines (Smartt, 1984; Waghmare and Mehra, 2000).

The member of family Fabaceae (Leguminoceae), is one of the most ancient originated and cultivated crop among all the crops in India (Deka and Sarkar, 1990). It grows and cultivate throughout tropical regions of Africa, central and South America, East and West Africa, Caribbean, Sudan, Egypt, China, Philippines, Indonesia and Malaysia. In India, it is cultivated in Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh and Maharashtra as a pulses, vegetable and forage. It had also been proved an excellent source of nitrogen fixer, to improve soil fertility, also useful as antioxidants also (Bradley, 1999).

Grass pea (*Lathyrus sativus* L.) is a crop of some economic value, in developing countries including India, Bangladesh, Nepal, and Ethiopia. The cultivation practices also noticed in China and countries of Europe, the Middle East, and Northern Africa. It serves a variety of purposes including food, feed and fodder owing in part to its nutritive qualities. In South Asian countries, grass pea is commonly grown for both grain and fodder purposes. Animal feed from *Lathyrus* is usually composed of ground or split grain or flour and is used primarily to feed lactating cattle or other draft dal. animals. Human diets include Lathyrus as grains that are boiled and then either consumed whole or processed for split In Indian Continent, Grass pea is a rabbi-season crop adapted to areas with arid or semiarid regions, is generally sown in October or November and harvested in February or March. Sowing date is largely determined by the time when the monsoon rains end, soil type and soil moisture profile during October.

Induction of mutation in recent times yielded some useful morphological variants and the some author successfully isolated and studied inheritance patterns of different flower colour, seed coat colour and stipule mutations in grass pea (Talukdar *et al*., 2001a; Talukdar and Biswas, 2005a, b). The main aim of present investigation is to inquire the effect of different doses of chemical as well as physical mutagens on chlorophyll frequency, its spectrum and leaf morphological changes in Lathyrus.

MATERIALS AND METHODS

The Lathyrus seeds variety of 'Mahatherara' was procured from the Indira Gandhi Krishi Vishvavidyalaya, Raipur (C.G.) India, used and treated by various doses of Chemical viz. EMS, SA, NMU and physical mutagen Gamma rays **[**Co^{60} gamma source (irradiation source capacity to release 3000 Ci delivery 7200 r/min curie), irradiated at the Department of Biophysics, Government Institute of Science, Aurangabad. (M.S.) India].

300 (for three replication) healthy and uniform size of seeds were pre-soaked in distilled water for 6 hours, for each dose and concentration, then theses seeds were immerse in the chemical mutagenic solution for 6 hours, with the different concentration 0.05, 0.10 and 0.15% for EMS; 0.05, 0.010 and 0.015 % for SA and 0.15, 0.30 and 0.45% for NMU, while for the physical mutagen the dose applied 15, 30 and 45 KR.

After the each chemical treatment, the treated seeds were washed thoroughly for 1h in running tap water to terminate the residual effect of the mutagenic chemicals. After the completion of the treatment the treated seeds were sown immediately in the field along with their respective controls to rise the M1 generation in a completely randomized block design (CRBD) with three replications. All the treatments including the controls were raised adopting a spacing of 30 cm in between rows and 15 cm in between plants. The experiment was conducted during Rabi (winter) season of 2017. Recommended agronomic practices were employed for field preparation, sowing and subsequent management of the Lathyrus crop.

The classification and identification of the chlorophyll mutants was done based on the nomenclature adopted by Gustafson (1940). The various chlorophyll deficient sectors and leaf abnormalities were noted dose wise separately. The Mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak *et al.* (1965).

$$\text{Mutagenic effectiveness (Physical mutagens)} = \frac{\text{Mf} \times 100}{\text{K rad.}}$$

$$\text{Mutagenic effectiveness (Chemical mutagens)} = \frac{\text{Mf} \times 100}{\text{c} \times \text{t}}$$

RESULTS AND DISCUSSION

The induction of chlorophyll deficient sectors observed in all doses of mutagen. Such sectors were albino (white), xantha (yellow), viridis (dull green) and chlorina (light green) types. They were distributed in the leaf lamina. It had been observed that the various doses of the mutagens EMS, SA, NMU and Gamma rays had proved effective inducing the chlorophyll deficient sectors in Lathyrus seeds (Table 1 and 2). The frequency of chlorophyll deficient sector of plants was noticed highest by EMS and Gamma rays treatment, as compared to other mutagens. The frequency of chlorophyll deficient sectors of Lathyrus plants ranged from 1.74 to 3.14% by EMS treatments, from 0.93 to 2.23% by SA treatments, 0.89 to 2.45% by NMU and 1.56 to 3.42% by Gamma ray treatments. Result shows the maximum chlorophyll and viable mutation frequency in all the treated seeds of Lathyrus.

Table1. Effect of mutagens on frequency of plants carrying chlorophyll deficient sectors in M_1 generation of *Lathyrus sativus* L.

Treatment	Dose (%) / (KR)	Frequency of chlorophyll Deficient Sectors%	±SE
Control	--	--	--
EMS	0.05	1.74	0.62
	0.10	2.45	0.58
	0.15	3.14	0.82
SA	0.005	0.93	0.95
	0.010	1.75	0.76
	0.015	2.23	0.88
NMU	0.15	0.89	0.67
	0.30	1.73	0.59
	0.45	2.45	1.02
Gamma rays	15	1.56	0.89
	30	2.38	0.96
	45	3.42	0.73

Figure1. Effect of mutagens on frequency of plants carrying chlorophyll deficient sectors in M_1 generation of *Lathyrus sativus* L.

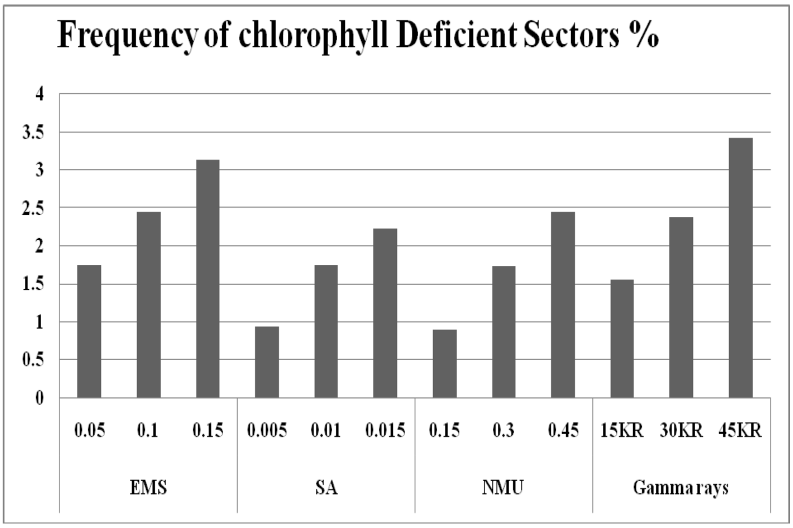
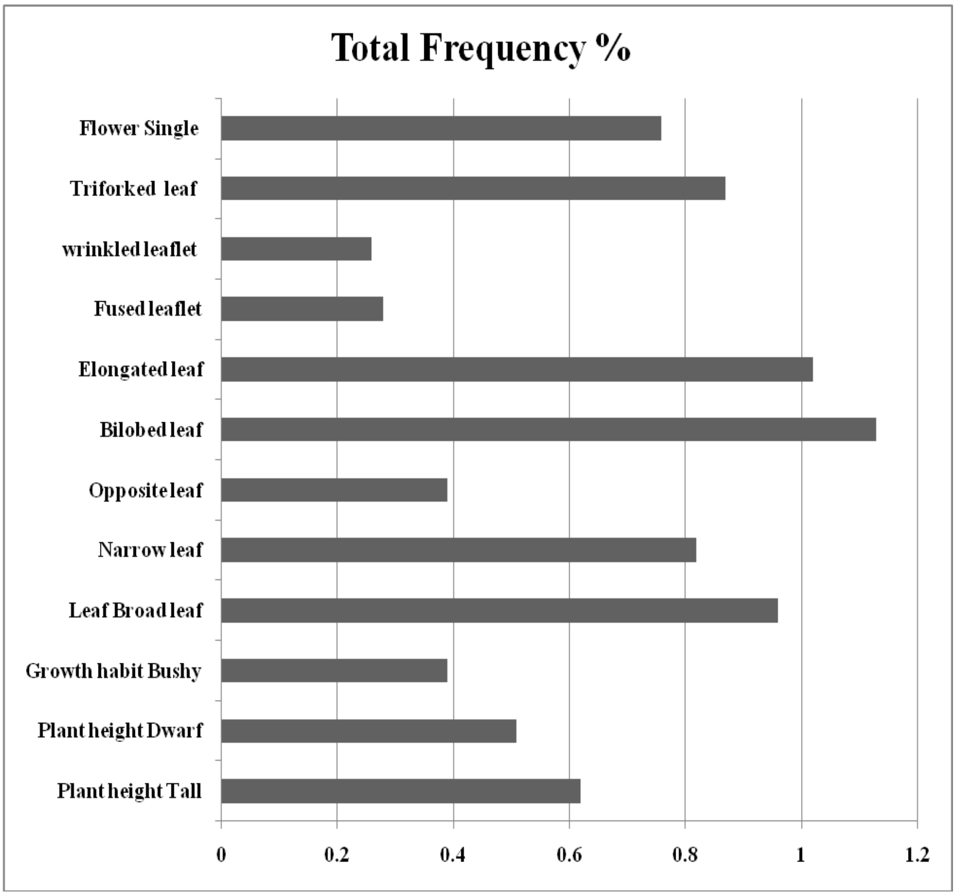


Figure 2. Effect of mutagens on total frequency percentage of leaf morphological abnormality observed in M_1 generation of *Lathyrus sativus* L.



The chlorophyll mutations was also recoded earlier by various researcher in Lentil (Sharma and Sharma, 1981), in Chickpea (Kharkwal 1998, Khan *et.al* 2005, Bara *et al.,* 2017, in *Lathyrus* (Prasad and Das, 1980 and Waghmare and Mehra, 2001), in *Vicia* beans (Bhat *et. al,* 2007), in Cowpea (Girija and Dhanavel, 2009 and Kumar *et al.,* 2010), in Black Gram (Thilagavathi and Mullainathan, 2009 ; Lal *et al*. 2009), in Mungbean (Kumar *et al.*, 2009), in green gram (Das and Baisakh, 2010), in Urdbean (Goyal and Khan, 2010), in *Trigonella* **(**Vasu and Hasan, 2011), in horse gram (Kulkarni and Mogle, 2013), in green gram (Vikram *et al.,* 2014), in *Lablab* (Monika and Seethraman, 2017 and Undirwade and Kulkarni, 2018). Chlorophyll mutation is shows the directly relation of the mutagenic effect. The occurrence of chlorophyll deficient mutant was noticed due to change in gene and a set of genes responsible for chlorophyll mutations. This conclusion is confirmed by the earlier reports of Kumar *et al*. (2009) in Mungbean, Monika and Seetharaman, (2017) in Hyacinth bean.

The seeds of Lathyrus treatment of various chemical concentration and physical mutagen doses was revealed the some abnormal shapes and size of leaves (Fig.1). The leaf variations comprised bilobed leaf (C), reduction in size of leaf (L), obtuse apex (A), retuse apex (B), notch at margin (C), sinuate leaf margin, tri apex leaflet (I), lanceolate (J) and wrinkled leaflet (L,N). The frequency of plants showed the leaf morphological changes observed in various doses of mutagens (Table 1 and Table 2).

Among the four mutagens, the EMS especially its 0.15% dose and Gamma rays 45KR succeeded in inducing the highest morphological

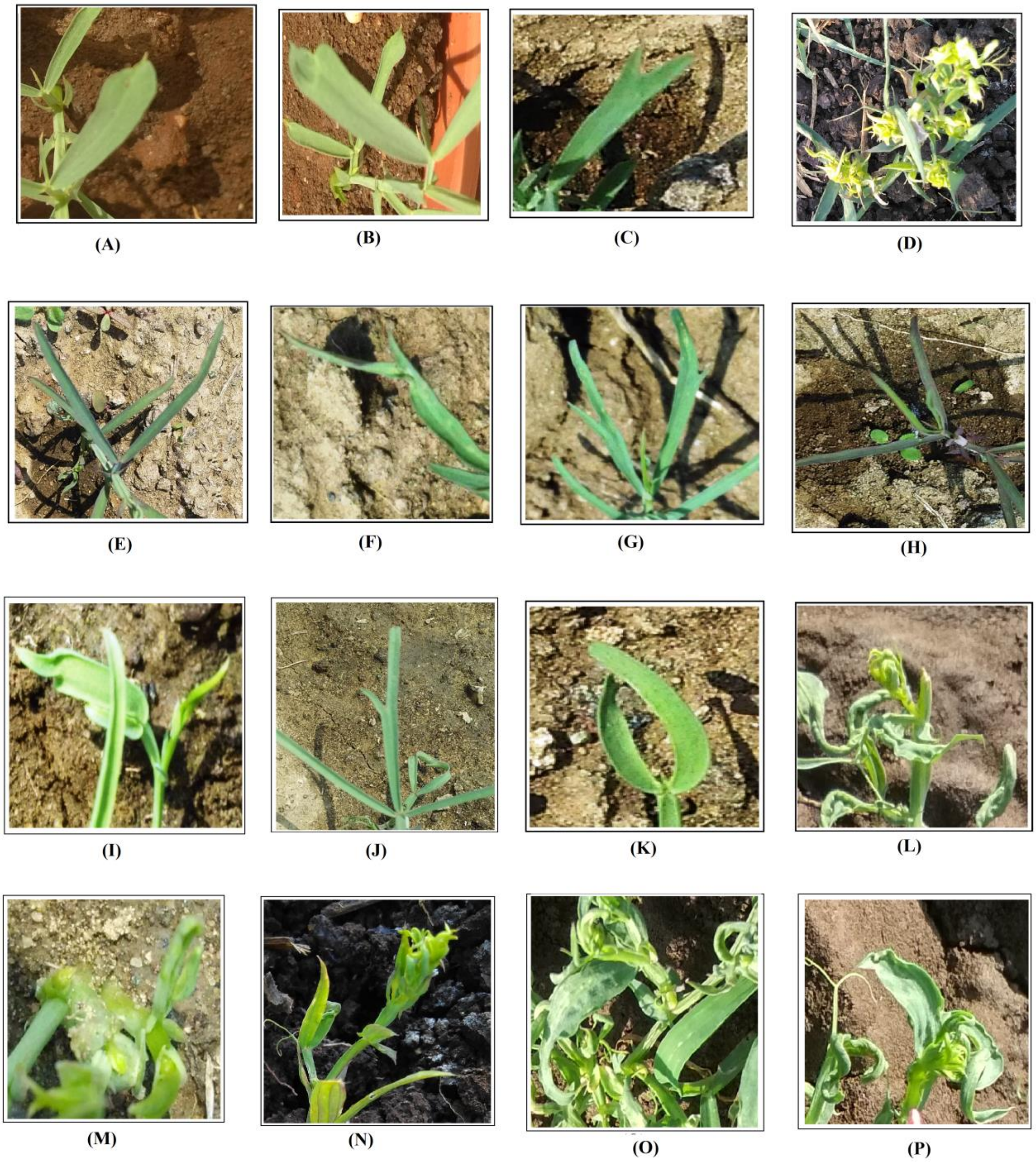


Figure1. Effect of of chemical and physical mutagens Chlorophyll deficient sectors in M_1 plants, morphological abnormality found in *Lathyrus sativus* L.

Figure Description: Frequency of Chlorophyll mutation: Albino (O), Xantha (D, M, N), Chlorina (A,B,P), Viridis (G,F).

Frequency of Phenotypic abnormality: Obtuse leaf apex(A), Acute leaf apex (B), Notch in center of leaf(C), bilobed leaf (C), reduction in size of leaf (L), retuse apex (B), notch at margin(C), sinuate leaf margin, tri apex leaflet (G, I), lanceolate (J) and wrinkled leaflet (L,O, P), Bilobed leaflet (E,F), Reduction in size of leaf (B,J), Wrinkled leaflet (L,O,P), Triforked leaf (G), Broad leaf (I,O,P), Opposite leaf with abnormal Shape (K).

frequency (3.14 and 3.42 %) of the leaf abnormalities, respectively. The lowest frequency of leaf abnormality changes carrying plants (0.93 % and 0.89 %) could be noted at 0.05% SA and 0.15 % in NMU, respectively.

In present investigation, leaf morphological changes may pertinent some abnormalities viz. reduction in size of leaves, various abnormal leaf shapes and sizes, observed in the M_1 generation mostly noticed in all treated dosesThe frequency of leaf morphological changes carrying plants showed correlation with increasing concentration of mutagen. The changes in leaf morphology had reported by some worker earliar, in Pea (Gelin, 1954), Soyabean (Zacharias, 1956), in Safflower Satpute (1994), in mungbean (Singh *et al.*, 2000 and Sangsiri *et al.*, 2005), Chickpea (More *et al.*, 2011) and French bean (Mahamune and Kothekar, 2012), in horse gram (Kulkarni and Mogle, 2013), in green gram (Vikram *et al.*, 2014), in *Lablab purpureus* (Monika and Seethraman, 2017) in chickpea (Bara *et al.*, 2017) in Hyacinth bean (Undirwade and Kulkarni, 2018). The disturbances in phytochromes causes due to abnormalities in leaves is such as, chromosomal aberrations, mitotic inhibition, disrupted auxin synthesis and mineral deficiencies, disturbance in DNA synthesis, enlargement of palisade, spongy and mesophyll tissues (Undirwade and Kulkarni, 2018). The formation of triforked leaves might be developed by the death of apical cells at meristematic zone which have specific influences on the development of leaves and leaf shape.

Table 2: Frequency (percent) and spectrum of morphological mutants induced by various mutagens in M1 generation of Lathyrus

Mutant type	EMS		SA		NMU		Gama rays		frequency	
	Conc.	Freq. (%)	Conc.	Freq. (%)	Conc.	Freq. (%)	Doses	Freq. (%)	Total	In all
Plant height Tall	0.05	0.08	--	--	0.15	0.06	15KR	0.11	0.25	0.62
	0.10	0.12	--	--	0.30	0.11	30KR	0.14	0.37	
Dwarf	0.10	0.06	0.010	0.13	--	--	15KR	0.07	0.26	0.51
	0.15	0.12	--	--	--	--	30KR	0.13	0.25	
Growth habit Bushy	0.10	0.02	0.010	0.15	0.15	0.03	15KR	0.04	0.24	0.39
	--	--	--	--	0.30	0.15	--	--	0.15	
Leaf Broad leaf	0.10	0.08	0.010	0.13	0.15	0.09	15KR	0.11	0.41	0.96
	0.15	0.11	0.015	0.12	0.30	0.17	30KR	0.15	0.55	
Narrow leaf	0.10	0.06	0.010	0.27	0.15	0.02	15KR	0.01	0.36	0.82
	0.15	0.09	0.015	0.19	0.30	0.12	30KR	0.06	0.46	
Opposite leaf	--	--	--	--	--	--	15KR	0.12	0.12	0.39
	0.15	0.02	0.015	0.08	--	--	30KR	0.17	0.27	
Bilobed leaf	0.10	0.07	0.010	0.1	0.15	0.1	15KR	0.21	0.48	1.13
	0.15	0.12	0.015	0.12	0.30	0.17	30KR	0.24	0.65	
Elongated leaf	0.10	0.04	0.010	0.19	0.15	0.13	30KR	0.06	0.42	1.02
	0.15	0.17	0.015	0.21	0.30	0.14	45 KR	0.08	0.6	
Fused leaflet	0.10	0.06	--	--	--	--	--	--	0.06	0.28
	0.15	0.14	0.015	0.04	--	--	30KR	0.04	0.22	
wrinkled leaflet	0.10	0.19	--	--	0.15	0.02	15KR	0.02	0.23	0.26
	--	--	--	--	0.30	0.03	30KR	--	0.03	
Triforked leaf	0.10	0.08	0.010	0.07	0.15	0.13	15KR	0.09	0.37	0.87
	0.15	0.1	0.015	0.11	0.30	0.17	30KR	0.12	0.5	
Single flower	0.10	0.09	0.010	0.01	0.15	0.12	30KR	0.12	0.34	
	0.15	0.12	0.015	0.03	0.30	0.14	45 KR	0.13	0.42	0.76

Conclusion:

The effects of chemical and physical mutagen in chlorophyll in Lathyrus are applied in identifying the genetic effect of mutagen. Mutations in the chlorophyll traits might be reflected in subsequent generations in the form of different mutants. The morphological frequency of chlorophyll deficient sectors induced in the present investigation included agronomical desirable characters which may possibly be utilized in future breeding programme.

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