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Gamma Rays and EMS Induced Chlorophyll Mutations in Grasspea (*Lathyrus sativus* L.)

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Abstract

The present investigation was undertaken at department of Genetics and Plant Breeding, BCKV, Mohanpur Nadia (W.B.). A comparative study of frequency and spectrum of chlorophyll mutations induced by Gamma rays (400, 500 and 600 Gy), EMS (0.5% and 1%) and their combination treatments (400Gy+0.5% and 400Gy+1%) in M_2 generation was done in three grasspea varieties *viz*., Nirmal, BioL-212 and Berhampur Local. The mutation frequency was estimated on M_2 seedling basis. Broad spectrum of chlorophyll mutations like Albino, Xantha, Albo-Xantha, Xanthalba, Albo-Viridis, Virescence, Chlorina, Maculata, Albescence and Tigrina types could be scored from all the three varieties in M_2 generation. Chlorophyll mutation frequency increases with increasing dose/ concentration of mutagen. Out of total chlorophyll mutation frequency (114.93%), Chlorina type exhibited maximum (17.93%) whereas Albescence type showed lowest frequency (2.16%). Total (Pooled) chlorophyll mutations frequency on variety basis indicated that out of total chlorophyll mutation frequency 43.06% (maximum) were produced in var. Nirmal, followed by BioL-212 (36.78%) and Berhampur Local (35.09%). Frequency of chlorophyll mutations on mutagen basis indicated that the gamma rays produced highest frequency (53.18%) followed by combined treatment of gamma rays and EMS (32.93%) where as chemical mutagen (EMS) produced lowest frequency (28.82%). The frequency and spectrum of chlorophyll mutations in grasspea are found to be both mutagen and variety dependent. The efficiency of different mutagens can be assessed through chlorophyll mutations which might be useful in selection of putative mutants in advanced generations.

Keywords: Grasspea, EMS, gamma rays, chlorophyll mutation

1. Introduction

The grasspea (*Lathyrus sativus* L.) is a versatile crop which used as food, feed and fodder, owing in part to its nutritive qualities (Mahler-Slasky and Kislev, 2010), belonging to the family Fabaceae, subfamily Papilionoideae, tribe Vicieae. It is also known as blue sweet pea, chickling vetch, Indian pea, Indian vetch, white vetch, almorta or alverjon (Spain), cicerchia (Italy), guaya (Ethiopia), and khesari in Bangladesh & India (Cruis, 2006). It has immense economic significance and cultivated as major crop especially in developing nations including India, Bangladesh, Pakistan, Nepal, and Ethiopia (Kumar et al., 2011 and Kumar et al., 2013) whereas, grown to a lesser extent in many countries of Europe, the Middle East, Northern Africa, as well as in South America. It is endowed with many

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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., 1989).

Induced and spontaneous mutations have been playing an important role in the origin of new cultivars of many Agricultural and Horticultural crops. Induced mutagenesis may be of special use in broadening the genetic variability of quantitatively inherited characters (Khan and Khan, 2011). Mutagenesis and mutation breeding can be a valuable supplement to conventional breeding methods. It can be used to create additional genetic variability that may be utilized by plant breeders in the development of cultivars for specific purposes or with specific adaptations (Rybinski, 2003). Genetic diversity which is the backbone of crop improvement may be introduced deliberately by employing ionizing radiation as well as chemical mutagens. Improvement in the frequency and spectrum of mutations in a predictable manner and thereby achieving desired plant characteristics for their either direct or indirect exploitation in the breeding programme is an important goal of mutation research (Patil et al., 2017). The scoring of chlorophyll mutations in M_2 generation has been proved to be the most dependable index for evaluating the genetic effects of mutagenic treatments (Gustaffson, 1951). Chlorophyll mutations are considered as the most dependable indices for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research (Wani et al., 2011). The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have been reported by Swaminathan et al., 1962; Solanki, 2005; Dhulgande et al., 2011; Bhosale and Hallale, 2011; Usharani and Kumar, 2015; Nair et al., 2016; Khursheed and Khan, 2016; More and Jagtap, 2016 and Bind et al., 2016 in barley, lentil, pea, blackgram, urdbean, cowpea, faba bean, *Dolichos* bean and cowpea respectively.

The present investigations deal with the studies of frequency and spectrum of chlorophyll mutations induced in the three varieties of *Lathyrus sativus* L. (2n=14) by gamma radiation and EMS (both differential and combined treatment of EMS and 400 Gy gamma radiation). Few workers like Prasad and Das (1980), Tripathy et al. (2012) and Ramezani and More (2014) have also worked on *Lathyrus sativus* but on varieties other than one included in the present investigations.

2. Materials and Methods

Three grasspea (*Lathyrus sativus* L.) varieties viz., Nirmal, BioL-212 and Berhampur Local comprised the experimental materials which were treated with Gamma rays, EMS and their combination treatments. 300 dry and dormant seeds for each treatment were irradiated with different doses of gamma rays (400, 500 and 600 Gy). Irradiations were done at the UGC- DAE consortium for scientific research, Kolkata centre (South campus of Jadavpur University, salt lake, Kolkata). The source of gamma rays was ^{60}Co and the dose rate was 7.12 Gy/minute. Similarly, the same number of seeds were pre-soaked in distilled water for 9 h, and subjected to chemical mutagens, ethylmethane sulphonate (Sigma Chemical Company, USA). Two different concentrations (0.5% and 1%) of EMS were used for chemical treatment. All solutions of the chemical mutagens were prepared in phosphate buffer having pH-7. For combination treatments, 300 seeds each were first irradiated with 400 Gy of gamma rays and then followed by the two EMS concentrations (0.5% and 1%). Following these the EMS treated seeds were thoroughly washed with running water for 1 hour to remove 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 M_1 generation at the B.C.K.V. farm, (W.B.) during year 2012-13. The seeds were sown in a randomized block design (RBD) with three replications having 100 seeds each for every treatment in case of each variety. Each M_1 plant was harvested separately and M_2 generation (2013−14) was raised from the composite sample of 25 seeds obtained from each M_1 harvested plant of a treatment. The M_2 population was evaluated in RBD with three replications, each replication plot consisting of 100 seeds and in addition to that 600 seeds of each treatment in all the three varieties were also raised in simple row method. The treated as well as control populations were carefully screened for various chlorophyll mutations. The identification and classification of the chlorophyll mutants was done based on the nomenclature adopted by Gustafson (1940). The mutation frequency was estimated on M_2 seedling basis.

3. Results and Discussion

3.1. Frequency of chlorophyll mutation

Frequency of chlorophyll mutations in the three varieties of grasspea were scored on M_2 seedling basis (Table 1) and it has been found that chlorophyll mutation frequency increases with increasing dose/ concentration of mutagen in all the varieties (Figure 1) except in Berhampur Local, where 600 Gy showed lower frequency (6.299%) than the 500 Gy (6.667%). However in case of chemical treatment (EMS), 1% EMS was found to be more effective than the two lower doses (400 and 500 Gy) of Gamma rays in variety Nirmal and BioL-212 but not in var. Berhampur Local where it was more effective than the

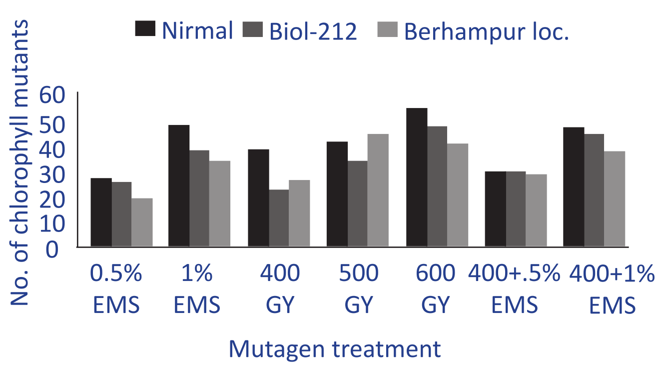

Figure 1: Treatment wise number of chlorophyll mutants in three varieties of grass pea

Table 1: Spectrum and frequency of different types of chlorophyll mutations in M_2 generation in Grasspea (*Lathyrus sativus* L.)

Treatment	No. of M_2 plants screened	Different chlorophyll mutation (%)										Total mutation frequency (%)
		A	X	AX	XA	AV	V	C	AS	T	M*	
Variety Nirmal												
Control	755											
0.5% EMS	658	0.304	0.456	0.152	0.000	0.304	0.304	0.760	0.000	0.760	0.912	3.951
1% EMS	660	0.000	0.758	0.606	0.455	0.758	1.061	1.212	0.303	0.758	1.212	7.121
400 Gy	652	0.460	0.307	0.000	0.613	0.767	0.920	0.613	0.153	1.227	0.613	5.675
500Gy	645	1.085	0.930	0.465	0.620	0.775	0.620	0.465	0.155	0.310	0.930	6.357
600 Gy	648	1.235	1.389	1.080	0.772	0.000	1.389	1.080	0.000	0.926	0.463	8.333
400+.5% EMS	635	0.472	0.945	0.472	0.630	0.000	0.472	0.787	0.000	0.472	0.315	4.567
400+1% EMS	652	0.613	1.074	0.767	0.767	0.920	0.920	1.380	0.153	0.000	0.460	7.055
Variety BioL-212												
Control	740											
0.5% EMS	646	0.000	0.310	0.464	0.000	0.310	0.619	0.774	0.000	0.619	0.774	3.870
1% EMS	635	0.472	0.630	0.787	0.472	0.787	0.630	0.630	0.000	0.787	0.630	5.827
400 Gy	628	0.637	0.637	0.637	0.000	0.000	0.478	0.955	0.159	0.000	0.000	3.503
500Gy	643	0.778	0.467	0.000	0.467	0.311	0.933	0.933	0.156	0.622	0.467	5.132
600 Gy	657	0.457	0.913	0.913	0.761	0.913	1.370	0.609	0.152	0.457	0.609	7.154
400+.5% EMS	634	0.946	0.631	0.000	0.158	0.789	0.000	1.104	0.158	0.789	0.000	4.574
400+1% EMS	655	0.763	1.069	0.763	0.458	0.916	0.458	1.374	0.305	0.305	0.305	6.718
Variety Berhampur Local												
Control	725											
0.5% EMS	662	0.302	0.000	0.604	0.000	0.302	0.604	0.453	0.000	0.453	0.000	2.719
1% EMS	638	0.470	0.470	0.784	0.313	0.784	0.940	0.470	0.000	0.627	0.470	5.329
400 Gy	640	0.469	0.625	0.313	0.000	0.625	0.781	0.469	0.000	0.313	0.469	4.063
500Gy	660	1.212	0.758	0.606	0.758	0.000	0.758	1.061	0.152	0.455	0.909	6.667
600 Gy	635	1.102	0.945	0.315	0.630	0.315	0.787	1.260	0.157	0.000	0.787	6.299
400+.5% EMS	645	0.620	0.620	0.000	0.930	0.465	0.620	0.620	0.000	0.000	0.465	4.341
400+1% EMS	652	0.767	0.920	0.613	0.000	1.074	0.460	0.920	0.153	0.613	0.153	5.675

*A: Albino; X: Xantha; AX: Albo- xenthia; XA: Xanthalba; AV: Albo-Vridis; V: Virescence; C: Chlorina; AS: Albescence; T: Tigrina; M: Maculata

400 Gy of Gamma rays only (Table 1). Combination treatment of two EMS concentrations with lower dose of Gamma rays showed increase in chlorophyll mutation frequency with increasing dose of chemical treatment, which indicates that different concentration of EMS with Gamma rays shows cumulative action. So after 600 Gy of Gamma rays 1% EMS concentration induces maximum chlorophyll frequency than the rest two doses (400 and 500 Gy) of Gamma rays indicating their greater effectiveness. Higher frequency and a wider spectrum of chlorophyll mutants in chemical mutagen EMS have been reported by Bhattacharya (2003); Sharma and Sharma (1984); Marki and Bianu (1970); Kawai and Sato (1969) in carnation, lentil, flax and rice, respectively.

3.2. Spectrum of chlorophyll mutations

Spectrum of chlorophyll mutation in three varieties of Lathyrus consisted of ten different types of chlorophyll mutations *viz.* Albino (A), Xantha (X), Albo-Xantha (AX), Xanthalba (XA), Albo-Viridis (AV), Virescence (V), Chlorina (C), Maculata (M), Albescence (AS) and Tigrina (T) (Figure 2). Although the

Figure 2: Different types chlorophyll mutations: (a) Albino, (b) Xantha, (c) Albo- xenthia, (d) Xanthalba, (e) Albo-Vridis, (f) Virescence, (g) Chlorina, (h) Maculata, (i) Albescence, (j) Tigrina

Xanthalba type seedlings died within fifteen days, but here they survived till flowering and set few seeds also in var. Nirmal and Berhampur Local with 400 Gy and 400 Gy + 0.5% EMS treatment, respectively. Among the mutagens individual higher frequency of different chlorophyll mutations were produced by gamma rays (Figure 3) except Albo-vridis where

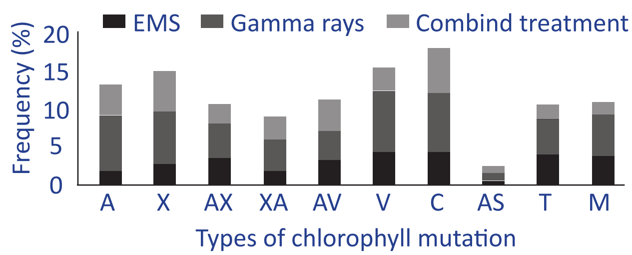

Figure 3: Frequency (%) of chlorophyll spectrum with respect of different mutagens in three varieties of grass pea

combination treatment (4.16%) showed higher frequency than the gamma rays (3.71%). However, among the varieties, Nirmal exhibited the higher frequency of all the chlorophyll mutation individually (Figure 4) excluding the Albino, Albo-Vridis and Albescence.

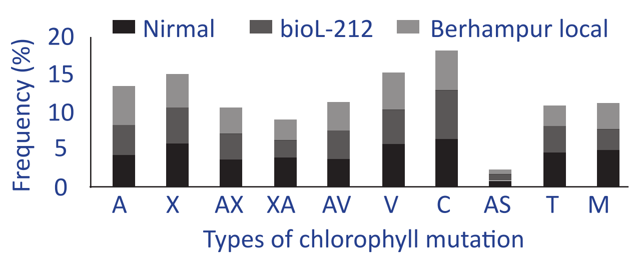

Figure 4: Frequency (%) of chlorophyll spectrum in three varieties of grass pea

A perusal of the Table 1 revealed that higher dose of gamma rays and combined treatment of mutagens produced the highest frequency of Albino, Xantha, Xanthalba, Albo-viridis, and Chlorina in all the varieties. Albo- xantha and Virescence exhibited higher frequency at 600 Gy in variety Nirmal and BioL-212 and at 1% EMS in var. Berhampur Local. Higher frequency of Maculata was observed in chemically treated population in var. Nirmal and Biol-212 and in 500 Gy treatment of var. Berhampur Local. But in case of Tigrina higher frequency was not the mutagen specific and it varied with variety. Among the all chlorophyll mutations, Chlorina and Albescence type was found to be with highest and lowest frequency respectively in all the three varieties (Figure 4) and it has been noticed that combination treatment of mutagens was more effective in producing Albescence type of chlorophyll mutation. The maximum induction of Chlorina type mutations in all the three varieties suggests that genes responsible for this mutation are readily available for mutagenic action.

Out of total (114.93%) chlorophyll mutation frequency, 13.17% belongs to Albino type, 14.85% to Xantha type, 10.34% to Albo-xantha type, 8.8% to Xanthalba type, 11.11% to Albo-vridis type, 15.13% to Virescence type, 17.93 % to Chlorina type (maximum), 2.16% to Albescence type (minimum), 10.49 % to Tigrina type and 10.94 % to Maculata type (Table 2).

Total (Pooled) chlorophyll mutations frequency on variety basis (varieties pooled over treatments) indicated that all the three varieties were found to respond the mutagenic treatments differentially and out of total chlorophyll mutation frequency 43.06% (maximum) were produced in var. Nirmal, 36.78% in var. BioL-212 and 35.09% in var. Berhampur Local. Frequency of chlorophyll mutations on mutagen basis (mutagens pooled over varieties) indicated that the individual mutagen and their combination treatment showed variable degree of chlorophyll mutation where physical mutagen (gamma rays) produced highest frequency (53.18%) followed by combined treatment of Gamma rays and EMS (32.93%) where as lowest frequency (28.82%) produced by chemical mutagen.

The frequency and spectrum of chlorophyll mutations are both mutagen and variety dependent in grasspea. Var. Nirmal, is more susceptible to both physical and chemical mutagens than the rest two varieties whereas var. Berhampur Local showing more resistant to chemical mutagens than the var. Nirmal and BioL-212 and to physical mutagen than the var. Nirmal only. Differences among the three varieties in respect of chlorophyll mutations, induced by mutagens may be due to genotypic differences existing among the varieties. Genetic differences even of a single gene induce significant changes in mutagen sensitivity, which influence not only the rate but also the spectrum of recoverable mutations (Kaul and Bhan, 1977).

Table 2: Total (pooled) frequency and spectrum of different types of chlorophyll mutants induced in M_2 generation of Grasspea (*Lathyrus sativus* L.)

Varieties/ Mutagen	Total no. of M_2 seedlings	Relative frequency (%) of chlorophyll spectrum										Total frequen-cy (%)
		A	X	AX	XA	AV	V	C	AS	T	M*	
Variety basis (varieties pooled over treatments)												
Nirmal	4550	4.170	5.858	3.543	3.857	3.524	5.687	6.299	0.765	4.453	4.906	43.059
BioL-212	4498	4.053	4.656	3.565	2.316	4.026	4.488	6.379	0.930	3.579	2.785	36.778
Berhampur local	4532	4.943	4.338	3.235	2.631	3.565	4.951	5.253	0.462	2.461	3.254	35.092
Total	13580	13.166	14.852	10.343	8.804	11.114	15.126	17.931	2.157	10.493	10.944	114.930
Mutagen basis (mutagens pooled over varieties)												
EMS	3899	1.549	2.623	3.398	1.240	3.244	4.158	4.299	0.303	4.004	3.998	28.818
Gamma Rays	5808	7.434	6.970	4.329	4.620	3.706	8.036	7.445	1.084	4.309	5.247	53.182
Combind treatment	3873	4.183	5.259	2.616	2.943	4.164	2.931	6.186	0.770	2.180	1.699	32.930
Total	13580	13.166	14.852	10.343	8.804	11.114	15.126	17.931	2.157	10.493	10.944	114.930

4. Conclusion

Most of the chlorophyll mutants are lethal in nature and hence do not have any economic value, therefore such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutants in the segregating generations, which might be further utilization in crop improvement programmes.

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