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Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus* L.

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Abstract

The spectrum and frequency of chlorophyll mutations was assessed in M_2 generation in P27, an improved cultivar of *Lathyrus sativus* with a range of gamma rays (50, 100, 150, 200, 250, 300, 350 and 400 Gy) and ethyl methane sulphonate [EMS 0.5% (2h & 4h), 1% (2h & 4h)] doses. Both, gamma rays and EMS induced a wider spectrum of chlorophyll mutations. Certain chlorophyll mutations such as chlorina, followed by chlorotica and xantha were found more frequently than others, indicating preferential induction of certain type of mutations. Fairly high frequency of chlorophyll mutants was obtained with EMS than in gamma rays. Dose dependent increase in chlorophyll mutation rate was observed based on plant population and segregating progenies in M_2 generation. Majority of segregating progenies yielded only one type of chlorophyll mutation and with an increase in the number of mutational events frequency of segregating families reduced. Both mutagenic effectiveness and efficiency were higher at lower doses of the mutagen. Mutagenic effectiveness, a measure of the frequency of mutations induced per unit dose of mutagen, and mutagenic efficiency, proportion of mutations in relation to undesirable effects, were higher with EMS treatments indicating EMS as more effective and efficient mutagen than gamma rays.

Key Words : Grasspea, chlorophyll mutations, mutagenic effectiveness, mutagenic efficiency

Introduction

In mutagenesis experiments, most of the mutations are lethal, semi-lethal or have very low productivity and do not have direct practical value. However, viable macro-mutations, though induced quantitatively very few, would be more valuable in genetic studies since, plants with altered characteristics or phenotype can serve as markers of the mutability of a variety or species. Frequency of such mutations also serves as an index of mutagenic sensitivity of various mutagenic agents and their dose effects. Majority of agricultural crop plants exhibit a high percentage of chlorophyll mutations in mutagenesis experiments. In fact, spectrum and frequency of chlorophyll mutations have been studied in great detail and approximately 250-300 genes responsible for chlorophyll mutations in barley have been determined [1, 2]. Wettstein *et al.* [3] have assigned about 198 recessive lethal chloroplast mutants to more than 80 loci, and conjectured that for the albina mutant alone several hundred genes might be responsible.

The chlorophyll mutation rate is conveniently being used as preliminary index of effectiveness (mutations per unit dose) of mutagens and mutability of the variety which in turn could be helpful to realize spectrum of desirable mutations in the treated populations. The present paper deals with the spectrum of chlorophyll mutations and frequency of occurrence of each type as a function of the mutagen. It also attempted to determine chlorophyll mutation rate including the families segregating for one or more types of chlorophyll mutations, mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate (EMS) in the grain legume *Lathyrus sativus.*

Materials and methods

Dry uniform size seeds with 10 per cent moisture of P27, an improved cultivar of *Lathyrus sativus,* were treated with 50, 100, 150, 200, 250, 300, 350 and 400 Gy gamma rays doses and also with freshly prepared 0.5 and 1.0 per cent aqueous solution of EMS for 2 and 4 hours each with intermittent shaking. The gamma rays and EMS treated materials along with untreated seeds of P27 as control were sown in *rabi* 1991-92 season at I.A.R.I., New Delhi.

Survival of plants was recorded at the time of maturity and was expressed as percentage of control. Mean number of seeds produced per pod was determined on ten randomly selected pods from each M_1 plant in all treatments and seed sterility was

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expressed as reduction in number of seeds per pod in relation to control. Selfed seeds of individual M_1 plants were harvested separately and grown as single plant progeny in M_2 generation in *rabi* season at I.A.R.I., New Delhi. Chlorophyll mutations were scored from crop emergence till the age of four weeks of seedlings. The spectrum of chlorophyll mutations was studied as per the modified scheme of Lamprecht [4]. The frequency of chlorophyll mutations was estimated based on M_2 plant population as well as percentage of segregating M_2 plant progenies in each treatment. Both mutagenic effectiveness and efficiency were determined as per the formulae suggested by Konzak *et al.* [5].

Results and discussion

From the relative frequency and spectrum of chlorophyll mutations produced in each treatment (Table 1) it is evident that both gamma rays and EMS induced a wide spectrum of chlorophyll mutations in *Lathyrus sativus*. The frequency of occurrence of chlorina mutants was relatively high in both mutagens followed by chlorotica and xantha. Among the various induced chlorophyll mutations, the relative range of frequency of each type of mutation was in the order - chlorina (22.77 to 50.0), chlorotica (12.19 to 39.74), xantha (3.39 to 13.64), albina (0.88 to 11.32), viro-maculata (0.53 to 10.0), xantha-virescence (1.96 to 6.52) and terminalis (0.88 to 3.66). Certain chlorophyll mutations (such as chlorina, chlorotica and xantha) occurred more frequently whereas chlorophyll mutations such as albo-virescence were not observed in M_2 population. Similarly, terminalis type of mutants induced very rarely. Nerkar [6] and Chekalin [7] also recorded occurrence of certain types of mutations more frequently than others in *Lathyrus sativus*. Occurrence of chlorina mutants in large number of crops have been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching due to deficiency of carotenoids [8].

In general, all mutagenic treatments induced fairly high frequency of chlorophyll mutations. Chlorophyll mutation rate did not show much variation with increase in gamma rays doses however, significantly higher mutation rate was observed with the increase in concentration and duration of EMS treatments. Thus, EMS showed a strong dose dependent relationship with respect to chlorophyll mutation frequency. Dose dependent increase in mutation frequency has also been reported in black gram [9], linseed [10] and lentil [11].

Mutation rate based on mutant M_2 progenies was comparable with the rate computed based on M_2 plants (Table 2). It is evident that the percentages of mutant progenies were comparatively higher with the lower doses of mutagen. Higher frequency of chlorophyll mutations with medium or lower doses of mutagen were reported by Srivastava *et al* [12] and Nadarajan *et al* [13] while, several other reports indicate dose dependent increase in mutation frequency [9-11]. The

Table 1. Spectrum of chlorophyll mutations in M_2 generation

Treatments	Total	Chlorophyll mutations (%)							
		Albina	Xantha	Chlorina	Xa.vir.	Chlo.vir.	Chlorotica	Terminalis	Maculata
Gamma rays									
50 Gy	82	6.10	24.39	32.93	3.66	9.76	12.19	3.66	7.32
100 Gy	53	11.32	20.75	28.30	3.77	7.54	22.64	1.89	3.77
150 Gy	46	4.34	15.22	41.30	6.52	10.86	15.22	-	6.52
200 Gy	22	4.55	13.64	31.81	-	13.64	27.27	-	9.90
250 Gy	4	-	25.00	50.00	-	-	25.00	-	-
300 Gy	20	5.00	25.00	30.00	5.00	-	25.00	-	10.00
350 Gy	36	8.33	22.22	27.78	5.55	-	25.00	2.78	8.33
400 Gy	-	-	-	-	-	-	-	-	-
Pooled	263	6.84	20.91	32.71	4.18	7.61	19.01	1.90	6.84
EMS									
0.5% (2h)	51	3.92	17.65	33.33	1.96	3.92	33.33	-	5.88
0.5% (4h)	127	8.66	16.54	39.37	-	9.45	19.69	1.57	4.72
1.0% (2h)	113	0.88	20.35	35.40	4.42	11.50	23.01	0.88	3.53
1.0% (4h)	78	-	14.10	33.33	2.56	3.85	39.74	2.56	3.85
Pooled	369	3.79	17.34	36.04	2.17	8.13	26.83	1.36	4.34
Overall	632	5.06	18.83	34.65	3.01	7.91	23.58	1.58	5.38

Xa. Vir. = Xantha-virescens, Chlo.vir. = Chlorina-virescens present study supports both the views in a way that gamma rays treatments do not show any kind of dose dependent relationship but EMS treatments do follow dose dependent relationship with respect to chlorophyll mutation rate computed based on M_2 plants as well as M_2 plant progenies. Reduction in mutation frequency at comparatively higher LD50 doses of mutagen may be due to higher chromosomal mutations, drastic reduction in survival and fertility, which might have contributed towards minimizing mutation frequency. The progeny or population size is also supposed to be another important factor in determining the mutation rate [2].

The majority of segregating progenies yielded only one type of chlorophyll mutation in all treatments and with an increase in the number of mutational events (two or more type of chlorophyll mutations) the frequency of segregating progenies decreased (Table 2). Mutations are known to alter nucleotide sequence of one or more genes (point mutations) which, in turn, affect specific activity or profile of one or several enzymes. Altered activity of enzymes/proteins has a definite bearing on growth, reproduction and survival. It can be assumed that multimutational events affect several genes and thus several enzymes/proteins, resulting into pleitropic effect. Most of the mutants bearing multimutational events thus may be lethal in the first generation, affecting the frequency of occurrence of multimutations in M_2 and further generations.

The overall induced chlorophyll mutation frequency for EMS and gamma rays indicate that EMS has produced higher chlorophyll mutations than the gamma rays. The average chlorophyll mutation rate computed based on M_2 plant population and M_2 progenies with gamma rays were 3.63 and 33.11 and with EMS treatments 7.11 and 56.7, respectively. However, the proportion of mutant progenies segregating for one or more type of chlorophyll mutant did not differ significantly in both physical as well as chemical mutagens.

Mutagenic effectiveness and efficiency were estimated based on the relative frequency of families segregating for chlorophyll mutations (Table 3). It was found that effectiveness and efficiency were higher at lower doses and decreased with the increase in dose of mutagens indicating negative relationship. Mutagenic effectiveness of EMS was as high as 45.53% in the range of 13.0 to 45.53% whereas for gamma rays it was 0.878 in the range from 0.044 to 0.878. An average effectiveness of (EMS) was several times higher than gamma rays. Similarly, mutagenic efficiency of EMS was also 3 to 4 times higher than that of gamma rays. Thus, chemical mutagen EMS was most effective and efficient in producing high proportion of mutations than gamma rays. Singh and Chaturvedi [14] observed that the combined treatment of chemical and physical mutagens were most effective than treatments using the mutagen separately.

Table 2. Frequency of chlorophyll mutations in M_2 generation

Treatments	M_2 plants			M_2 progenies			Progenies segregating for			
	Total	Mutants	% mutated plants	Total	Segre-gating	% mutant	1 type	2 types	3 types	4 types
Gamma rays										
50 Gy	2318	82	3.54	98	43	43.88	62.8	23.3	11.6	2.2
100 Gy	1813	53	2.92	96	35	36.46	54.3	37.1	5.7	2.9
150 Gy	1105	46	4.16	80	24	30.00	70.8	25.0	4.2	-
200 Gy	725	22	3.03	60	70	28.33	70.6	29.4	-	-
250 Gy	199	4	2.01	27	3	11.11	100.0	-	-	-
300 Gy	633	20	3.16	53	13	24.53	69.2	30.8	-	-
350 Gy	386	36	9.33	31	16	51.61	68.7	12.5	12.5	6.3
400 Gy	56	0	0.0	11	0	0.0	-	-	-	-
Pooled	7235	263	3.63	456	151	33.11	64.9	26.5	6.6	2.00
EMS										
0.5% (2h)	1411	51	3.61	75	34	45.53	79.4	14.7	2.9	2.9
0.5% (4h)	1325	127	9.58	75	46	61.33	52.1	34.8	10.9	2.2
1.0% (2h)	1581	113	7.14	75	51	68.00	62.7	29.4	7.8	-
1.0% (4h)	868	78	8.98	75	39	52.00	59.0	33.3	7.7	-
Pooled	5185	369	7.11	300	170	56.70	62.4	28.8	7.6	1.2
Overall	12420	632	5.08	756	321	42.46	63.5	27.7	7.2	1.6

Table 3. Mutagenic effectiveness and efficiency in *Lathyrus sativus* L.

Treatments	Percentage				Mutagenic efficiency	
	lethality (L)	seed sterility (S)	mutated families (MF)	mutagenic effective-ness MF/kR or Mf/tc	MF/L	MF/S
Gamma rays						
50 Gy	47.40	12.50	43.88	0.878	0.934	0.510
100 Gy	60.59	14.19	36.46	0.365	0.602	2.569
150 Gy	71.32	24.32	30.00	0.200	0.421	1.234
200 Gy	88.35	27.03	28.33	0.142	0.320	1.048
250 Gy	91.79	26.69	11.11	0.044	0.121	0.416
300 Gy	93.07	29.05	24.53	0.082	0.264	0.844
350 Gy	96.17	40.54	51.61	0.147	0.534	0.128
400 Gy	98.18	56.08	0.00	-	-	-
EMS						
0 5% (2h)	24.57	5.41	45.53	45.53	1.853	8.416
0.5% (4h)	52.98	21.62	61.33	30.67	1.158	2.837
1.0% (2h)	42.01	15.20	68.00	34.00	1.619	4.474
1.0% (4h)	59.48	25.68	52.00	13.00	0.874	2.045

t = duration of treatment with chemical mutagen,
c = concentration of chemical mutagen in percentage.

Both mutagenic effectiveness and efficiency were higher at lower doses of physical and chemical mutagen. Higher efficiency at the lower concentration of the mutagen appears mainly due to the fact that injury, lethality and sterility increases with an increase in the mutagen concentration than actual mutations [5, 15].

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