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Short Communication

Frequency and spectrum of chlorophyll mutations in *macrosperma* lentil (*Lens culinaris* Medik.)

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Uniform seeds of a *macrosperma* lentil cv., Precoz Selection (PS), were treated with three doses (5, 10 and 20 kR) of gamma rays and three doses (0.005, 0.01 and 0.02%) each of ethylene imine (EI) and N-nitroso-N-ethyl urea (NEU). The treated seeds along with control were sown immediately in the field to raise the M_1 generation. The M_1 material was classified into four groups on the basis of total biological damage to the plants expressed as leaf-aberrations (low/high number of a-sectors) at seedling stage and fertility reduction at maturity (low/high) as a consequence of mutagenic treatment. The four mutagenic damage groups, thus formed were low damage in the form of a-sectors + less fertility reduction (LL), high seedling damage + less fertility reduction (HL), low seedling damage + high sterility (LH) and high seedling damage + high sterility (HH). In M_2 generation, the treated progenies/plants and control (Table 1) were screened for lethal chlorophyll mutations from emergence till the age of four week, whereas viable chlorophyll mutations

Table 1. Frequency of chlorophyll mutations in M_2 generation

Treatment	Gamma rays						EI						NEU					
	Progenies			Plants			Progenies			Plants			Progenies			Plants		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Control	58	-	-	290	-	-	56	-	-	280	-	-	60	-	-	300	-	-
5 kR/0.005%																		
LL	57	1	1.8	805	1	0.12	72	2	2.8	1080	3	0.28	63	3	4.8	819	3	0.37
LH	60	2	3.3	854	2	0.23	74	3	4.1	1036	3	0.29	57	3	5.3	790	4	0.51
HL	56	3	5.4	763	3	0.39	74	4	5.4	1126	5	0.44	66	4	6.3	908	5	0.55
HH	60	4	6.7	796	5	0.63	70	5	7.1	1033	6	0.58	64	4	6.1	872	6	0.69
Overall	233	10	4.3	3218	11	0.34	290	14	4.8	4275	17	0.40	250	14	5.6	3389	18	0.53
10 kR/0.01%																		
LL	87	3	3.5	1218	3	0.25	84	3	3.6	1196	4	0.33	85	4	4.7	1095	8	0.73
LH	88	3	3.4	1144	4	0.35	88	5	5.7	1232	7	0.57	85	5	5.9	1115	9	0.81
HL	80	4	5.0	1113	4	0.36	87	6	6.9	1218	8	0.66	93	8	8.6	1296	11	0.85
HH	82	6	7.3	1142	7	0.61	86	7	8.1	1118	9	0.81	77	8	10.4	1024	12	1.20
Overall	337	16	4.7	4617	18	0.39	345	21	6.1	4764	28	0.59	340	25	7.4	4530	40	0.88
20 kR/0.02%																		
LL	94	2	3.2	1405	4	0.28	94	2	2.1	1316	3	0.23	86	3	3.5	1290	4	0.31
LH	98	5	5.1	1366	5	0.37	95	4	4.2	1425	5	0.35	88	5	5.7	1408	7	0.50
HL	83	5	6.0	1154	6	0.52	93	6	6.5	1302	7	0.54	105	7	6.7	1680	10	0.60
HH	86	6	7.0	1118	8	0.72	92	8	8.7	1375	10	0.73	104	8	7.8	1560	11	0.71
Overall	361	19	5.2	5043	23	0.46	374	20	5.3	5418	25	0.46	383	23	6.0	5938	32	0.54
Total mutations	931	45	4.8	12878	52	0.40	1009	55	5.4	14457	70	0.48	973	62	6.4	13857	90	0.65

A: Total number of progenies/plants evaluated; B : Number of mutated progenies/plants; C : Per cent mutated progenies/plants

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were scored throughout the life period of the plants. Mutation frequency was calculated as the percentage of mutated progenies and plants. Different kinds of chlorophyll mutations (albina, chlorina, xantha and viridis) were identified in accordance with the modified classification of Blixt [1].

In general, all three mutagens induced high frequency of chlorophyll mutations (5.5% and 0.51% mutated M_2 progenies and plants, respectively), whereas the untreated (control) material did not induce any chlorophyll mutation (Table 1). NEU was the most effective mutagen, inducing chlorophyll mutations in 6.3% M_2 progenies and 0.65% M_2 plants, followed by EI (5.4% and 0.48% mutated progenies and plants, respectively) and gamma rays (4.7% and 0.40%). Thus, the chemicals induced higher frequency of chlorophyll mutations than radiations (gamma rays). This is in agreement with earlier reports, lentil [2, 3], mungbean [5], grasspea [6] and chickpea [7]. The mutagens based on the frequency of chlorophyll mutations induced were observed in the following order: NEU > EI > gamma rays. Superiority of NEU to induce the highest frequency of chlorophyll mutations has been demonstrated in lentil [3]. The frequency of chlorophyll mutations induced was the lowest with gamma rays. Comparatively lower frequency of chlorophyll mutations with gamma rays has been reported by some workers [2, 4, 6, 7].

Dose-dependent relationship was observed in the case of gamma rays, i.e. with increase in dose there was corresponding increase in chlorophyll mutation frequency (5 kR < 10 kR < 20 kR), whereas for chemicals, the medium dose (0.01%) was found most effective. The highest mutation frequency was recorded with the medium dose (0.01%) of NEU (7.4% and 0.88% mutated progenies and plants, respectively). So far as radiations are concerned, the findings of the present investigation are in agreement with the observations in peas [2] and lentil [4]. However, Kharkwal [7] reported dose-dependent decrease in the frequency of chlorophyll mutations with gamma rays. For chemicals, the present investigation conforms the results of some earlier studies [5] where higher frequency of chlorophyll mutations with medium or low doses of mutagens were observed. It seems that the strong mutagens reach their saturation point even at lower doses in the highly mutable genotypes and further increase in dose does not add to the mutation frequency. With increase in dose beyond a point, the strong mutagens become more toxic than the higher doses of relatively weaker mutagens [5].

The highest frequency of chlorophyll mutations with all the mutagens and their doses was recorded in HH group and the lowest in the LL group. Among the intermediate groups, HL carried more chlorophyll mutations than LH. Different damage groups induced chlorophyll mutations in the order: HH > HL > LH > LL in all the mutagenic treatments. Analogous results have been reported in peas [7].

Four types of chlorophyll mutations, albina,

Table 2. Spectrum of chlorophyll mutations in M_2 generation

Treatment		Total no. of plants	Distribution of different chlorophyll mutations							
			Albina		Chlorina		Xantha		Viridis	
			No.	%	No.	%	No.	%	No.	%
Control		870	-	-	-	-	-	-	-	-
Gamma rays										
5 kR:	LL	805	-	-	-	-	1	0.12	-	-
	LH	854	-	-	1	0.11	1	0.12	-	-
	HL	763	-	-	1	0.13	2	0.26	-	-
	HH	796	1	0.13	1	0.12	2	0.25	1	0.13
	Overall	3218	1	0.03	3	0.09	6	0.19	1	0.03
10 kR:	LL	1218	-	-	-	-	2	0.16	1	0.08
	LH	1140	-	-	1	0.09	2	0.17	1	0.09
	HL	1113	-	-	1	0.09	3	0.27	-	-
	HH	1142	1	0.09	2	0.18	3	0.26	1	0.09
	Overall	4617	1	0.02	4	0.09	10	0.22	3	0.06
20 kR:	LL	1405	1	0.07	-	-	2	0.14	1	0.07
	LH	1366	-	-	1	0.07	2	0.15	2	0.15
	HL	1154	1	0.09	1	0.09	2	0.17	2	0.17
	HH	1118	1	0.09	2	0.18	3	0.27	2	0.18
	Overall	5043	3	0.06	4	0.08	9	0.18	7	0.14

Table 2 Contd.

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Treatment		Total no. of plants	Distribution of different chlorophyll mutations							
			Albina		Chlorina		Xantha		Viridis	
			No.	%	No.	%	No.	%	No.	%
Overall Gamma rays EI		12878	5	0.04	11	0.09	25	0.19	11	0.09
0.005%	LL	1080	-	-	-	-	1	0.09	2	0.18
	LH	1036	-	-	-	-	1	0.10	2	0.19
	HL	1126	-	-	1	0.09	2	0.18	2	0.18
	HH	1033	1	0.10	1	0.10	2	0.19	2	0.19
	Overall	4275	1	0.02	2	0.05	6	0.14	8	0.19
0.01%	LL	1196	-	-	-	-	1	0.08	3	0.25
	LH	1232	-	-	1	0.08	3	0.24	3	0.24
	HL	1218	1	0.08	1	0.08	2	0.16	4	0.33
	HH	1118	2	0.18	1	0.09	2	0.18	4	0.36
	overall	4764	3	0.06	3	0.06	8	0.17	14	0.29
0.02%	LL	1316	-	-	-	-	1	0.08	2	0.15
	LH	1425	-	-	2	0.14	1	0.07	2	0.14
	HL	1302	-	-	2	0.15	2	0.15	3	0.23
	HH	1375	1	0.07	2	0.15	3	0.22	4	0.29
	Overall	5418	1	0.01	6	0.11	7	0.13	11	0.20
Overall EI		14457	5	0.03	11	0.08	21	0.15	33	0.23
NEU										
0.005%	LL	819	-	-	1	0.12	1	0.12	1	0.12
	LH	790	-	-	1	0.13	2	0.25	1	0.13
	HL	908	-	-	1	0.11	2	0.22	2	0.22
	HH	872	1	0.11	1	0.11	2	0.23	2	0.23
	Overall	3389	1	0.03	4	0.12	7	0.21	6	0.18
0.01%	LL	1095	-	-	3	0.27	2	0.18	3	0.27
	LH	1115	1	0.09	3	0.27	2	0.18	3	0.27
	HL	1296	1	0.08	2	0.15	4	0.31	4	0.31
	HH	1024	1	0.10	2	0.20	4	0.39	5	0.49
	Overall	4530	3	0.07	10	0.22	12	0.26	15	0.33
0.02%	LL	1290	-	-	1	0.08	1	0.08	2	0.16
	LH	1408	-	-	2	0.14	2	0.14	3	0.21
	HL	1680	1	0.06	3	0.18	3	0.18	3	0.18
	HH	1560	1	0.06	3	0.19	3	0.19	4	0.26
	Overall	5938	2	0.03	9	0.15	9	0.15	12	0.20
Overall NEU		13857	6	0.04	23	0.17	28	0.20	33	0.24
Overall experiment		41192	16	0.04	38	0.11	74	0.18	77	0.19

chlorina, xantha and viridis were isolated with all three mutagens (Table 2). These four types of chlorophyll mutations [2, 4]; albina, xantha and chlorina [6, 7], and xantha and viridis [9] have been reported earlier in different crops. Certain chlorophyll mutation types appeared more frequently than others: viridis (0.19%), xantha (0.18%), chlorina (0.11%) and albina (0.04%). Singh [2] observed viridis to occur with maximum frequency followed by chlorina. However, Waghmare and Mehra [6], Kharkwal [7] and Vo [8] observed higher frequency of lethal mutations than nonlethal mutations. Such conflicting results by different workers are perhaps due to the differential response of different crops, genotypes and, most important, differences in dose range used in different studies.

In general, relative differences in mutability of genes for various chlorophyll mutations with different mutagens were observed. The viridis mutations appeared most frequently following NEU (0.24%) and EI (0.23%) treatments, whereas xantha appeared most frequently with NEU (0.20%) and gamma rays (0.19%), and chlorina with NEU (0.17%). Further, albina mutations appeared in higher proportion with gamma rays and NEU (0.04%), followed by EI (0.03%). Among all the mutations, albina appeared with the lowest frequency

with all the mutagens under study. The relatively poor induction of this mutation has been reported earlier [2, 4, 6], however, Kharkwal [7] reported the highest frequency of albina mutations. In general, the spectrum of chlorophyll mutations was not influenced by the groups of M_1 damage, except that some mutation types occurred more frequently than others (quantitative difference) in certain groups [2].

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