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Induced Chlorophyll Mutations in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

A broad spectrum of chlorophyll mutations was obtained in M_2 generation by using EMS, SA and HZ in two varieties viz; Avrodhi and BG-256 of chickpea (*Cicer arietinum* L.). Lower and moderate concentrations of EMS gave higher frequency of chlorophyll mutations whereas no such trend was noticed with other two mutagens. Total (pooled) chlorophyll mutation frequency was found maximum with EMS treatments followed by HZ and SA. 'xantha' followed by 'chlorina' out numbered other types of chlorophyll mutants in both the varieties. In both the varieties, the order of mutagenic effectiveness was: HZ>SA>EMS. Three criteria viz; seedling injury (Mf/I), pollen sterility (Mf/S) and meiotic abnormalities (Mf/Me) were taken into account for estimation of mutagenic efficiency. The order of efficiency in both varieties with regard to Mf/I and Mf/S was: HZ>EMS>SA and EMS>HZ>SA respectively, while with regard to chromosomal aberrations, the order of efficiency in var. Avrodhi was: EMS>SA>HZ and it was: HZ>EMS>SA in var. BG-256.

Key Words: Chickpea; Chlorophyll mutations; Mutagenic effectiveness and efficiency
Abbreviations: EMS = Ethyl methane sulphonate; SA = Sodium azide; HZ = Hydrazine hydrate

INTRODUCTION

Chickpea, an important pulse crop, is attracting the attention of plant breeders to reconstruct it genetically. A number of chemical mutagens have been found to enhance genetic variability in cultivated crops (Khan *et al.*, 2004). The mutagenic effect is reflected in the segregation of chlorophyll mutants, these mutations were evaluated in order to determine the effectiveness and efficiency of chemical mutagens in two varieties of chickpea. The results of this study are reported in this paper.

MATERIALS AND METHODS

Seeds of two chickpea (*Cicer arietinum* L.) varieties *viz*; Avrodhi and BG-256, were pre-soaked in distilled water for 9 h, were subjected to chemical mutagens, ethyl methane sulphonate (EMS), sodium azide (SA) and hydrazine hydrate (HZ) for 6 h at 25±1°C. The concentrations used for EMS ranged from 0.1-0.4% whereas these were 0.01-0.04% for SA and HZ. All solutions of the chemical mutagens were prepared in phosphate buffer of pH-7. Following these treatments, the seeds were thoroughly washed with running water and sown in the field along with untreated controls. Three replications of 100 seeds each were sown for every treatment in each variety to raise the M_1 generation. The M_2 generation was grown from single plant M_1 progeny seeds. The frequency and spectrum of the different chlorophyll mutants were scored in M_2. They were identified and classified according to Gustafsson (1940). Effectiveness and efficiency of the different treatments of mutagens were calculated by the method suggested by Konzak *et al.* (1965).

RESULTS

Frequency and spectrum of chlorophyll mutations. Five different types of chlorophyll mutants were obtained when seedlings were 8-20 days old. The spectrum of chlorophyll mutations included: *albina*, *chlorina*, *tigrina*, *viridis* and *xantha* in both the varieties (Tables I & II). *Chlorina* and *viridis* types survived up to maturity while the others died at seedling stage. A brief description of these chlorophyll mutants is given below.

Albina. The mutants were white, lethal, survived for upto 10-12 days.

Chlorina. These were characterized by the presence of light green colour of the seedling. Some of them died within 20 days. However, a few vigorous surviving plants flowered but did not bear fruits.

Tigrina. The leaves were yellow with green patches. The mutants survived for about 15 days.

Viridis. The mutants were characterized by reduced height and showed a dark green foliage colour. The plants were slow growing and had low seed yield.

Xantha. These mutants, predominant in the mutagenized population, were characterized by bright yellow colour of the seedlings. These seedlings survived for 10-20 days only.

A perusal of Tables I and II reveals that lower concentrations of EMS produced higher frequency of chlorophyll mutations whereas no such trend was observed in SA and HZ treatments in both the varieties. Out of the total (82.41%) chlorophyll mutation frequency, 37.03% belonged to 'xantha' type; 22.77% to 'chlorina' type; 10.48% to 'viridis' type; 6.89% to 'tigrina' type and 5.24% to 'albina' type (Table III). Total (Pooled) chlorophyll mutations frequency on mutagen basis (mutagens pooled

Table I. Frequency and spectrum of chlorophyll mutants in M_2 generation of chickpea (*Cicer arietinum* L.) var. Avrodhi

Treatment	No. of M_1 Plant Progenies	No. of M_2 seedlings	Relative frequency (%) of chlorophyll spectrum					Total Frequency (Mf %)
			Albina	Chlorina	Tigrina	Viridis	Xantha	
Control	60	834	--	--	--	--	--	--
0.1% EMS	60	821	0.73	1.95	0.73	0.97	2.80	7.18
0.2% EMS	60	780	0.51	1.41	1.02	0.77	1.92	5.63
0.3% EMS	60	732	0.14	0.95	0.40	0.68	0.82	2.99
0.4% EMS	60	691	--	0.58	--	0.72	0.87	2.17
0.01% SA	60	815	--	0.74	--	--	0.98	1.72
0.02% SA	60	750	0.13	0.80	0.27	0.40	1.20	2.80
0.03% SA	60	688	--	0.87	0.14	--	0.87	1.88
0.04% SA	60	645	--	0.31	--	0.46	0.77	1.54
0.01% HZ	60	831	0.12	0.36	--	0.24	0.48	1.20
0.02% HZ	60	799	0.25	0.75	0.37	0.62	1.63	3.62
0.03% HZ	60	723	0.28	2.49	0.41	0.55	3.73	7.46
0.04% HZ	60	701	0.14	1.14	0.57	0.57	1.85	4.27

Table II. Frequency and spectrum of chlorophyll mutants in M_2 generation of chickpea (*Cicer arietinum* L.) var. BG-256

Treatment	No. of M_1 Plant Progenies	No. of M_2 seedlings	Relative frequency (%) of chlorophyll spectrum					Total Frequency (Mf %)
			Albina	Chlorina	Tigrina	Viridis	Xantha	
Control	60	849	--	--	--	--	--	--
0.1% EMS	60	840	0.36	1.67	0.59	0.59	2.38	5.59
0.2% EMS	60	815	0.49	1.23	0.61	0.86	3.07	6.26
0.3% EMS	60	786	0.13	0.51	0.25	0.51	1.40	2.80
0.4% EMS	60	735	--	0.68	--	0.27	0.95	1.90
0.01% SA	60	829	--	0.24	0.12	--	0.60	0.96
0.02% SA	60	797	0.12	0.38	--	0.12	0.75	1.37
0.03% SA	60	732	0.14	0.55	0.14	0.27	0.82	1.92
0.04% SA	60	679	--	0.59	--	--	0.88	1.47
0.01% HZ	60	843	0.19	0.59	0.24	0.12	0.83	1.97
0.02% HZ	60	829	0.60	0.96	0.24	0.72	1.81	4.33
0.03% HZ	60	788	0.63	1.78	0.51	0.76	3.55	7.23
0.04% HZ	60	723	0.28	1.24	0.28	0.28	2.07	4.15

Table III. Total (pooled) frequency and spectrum of different types of chlorophyll mutants induced in M_2 generation of chickpea (*Cicer arietinum* L.)

Mutagen / Varieties	Total No. of M_2 seedlings	Relative frequency (%) of chlorophyll spectrum					Total Frequency (%)
		Albina	Chlorina	Tigrina	Viridis	Xantha	
Mutagen basis (mutagens pooled over varieties)							
EMS	6200	2.36	8.98	3.60	5.37	14.21	34.52
SA	5935	0.39	4.48	0.67	1.25	6.87	13.66
HZ	6237	2.49	9.31	2.62	3.86	15.95	34.23
Total	18372	5.24	22.77	6.89	10.48	37.03	82.41
Variety basis (varieties pooled over treatments)							
Avrodhi	8976	2.30	12.35	3.91	5.98	17.92	42.46
BG-256	9396	2.94	10.42	2.98	4.50	19.11	39.95
Total	18372	5.24	22.77	6.89	10.48	37.03	82.41

over varieties) indicated almost equal frequency in EMS (34.52%) and HZ (34.23%) while SA showed lowest frequency (13.66%). Frequency of chlorophyll mutations on variety basis indicated that both the varieties were found to respond the mutagenic treatments differently. Out of the total chlorophyll mutation frequency, 42.46% were produced in var. Avrodhi and 39.95% in var. BG-256.

Mutagenic effectiveness and efficiency. Data on effectiveness and efficiency for EMS, SA and HZ in varieties Avrodhi and BG-256 are presented in (Tables IV

Table IV. Effectiveness and efficiency of the mutagens in M_2 generation of chickpea (*Cicer arietinum* L.) var. Avrodhi

Treatment	% Seedling injury (I)	% Pollen sterility (S)	% Meiotic abnormalities (Me)	% Mutation frequency (Mf)	Mutagenic Effectiveness (Mf/t.c.)	Mutagenic efficiency		
						Mf/I	Mf/S	Mf/Me
0.1% EMS	18.32	12.65	3.18	7.18	11.97	0.39	0.57	2.26
0.2% EMS	26.96	21.15	4.32	5.63	4.69	0.21	0.27	1.30
0.3% EMS	35.70	28.87	8.60	2.99	1.66	0.08	0.10	0.35
0.4% EMS	51.12	42.57	13.04	2.17	0.90	0.04	0.05	0.17
Overall	33.02	26.31	7.28	4.49	4.80	0.18	0.25	1.02
0.01% SA	10.54	9.39	1.77	1.72	28.66	0.16	0.18	0.97
0.02% SA	15.06	17.48	3.10	2.80	23.33	0.18	0.16	0.90
0.03% SA	26.38	25.72	4.20	1.88	10.44	0.07	0.07	0.45
0.04% SA	37.19	31.93	6.06	1.54	6.45	0.04	0.05	0.25
Overall	22.29	21.13	3.78	1.99	17.22	0.11	0.11	0.64
0.01% HZ	11.85	10.09	3.23	1.20	20.00	0.10	0.12	0.37
0.02% HZ	15.42	20.02	6.92	3.62	30.16	0.23	0.18	0.52
0.03% HZ	24.70	31.88	9.59	7.46	41.44	0.30	0.23	0.78
0.04% HZ	32.30	38.96	12.29	4.27	17.79	0.13	0.11	0.35
Overall	21.06	25.23	8.00	4.14	27.34	0.19	0.16	0.50

Table V. Effectiveness and efficiency of the mutagens in M_2 generation of chickpea (*Cicer arietinum* L.) var. BG-256

Treatment	% Seedling injury (I)	% Pollen sterility (S)	% Meiotic abnormalities (Me)	% Mutation frequency (Mf)	Mutagenic Effectiveness (Mf/t.c.)	Mutagenic efficiency		
						Mf/I	Mf/S	Mf/Me
0.1% EMS	13.04	10.19	4.50	5.59	9.32	0.43	0.55	1.24
0.2% EMS	21.26	18.16	5.94	6.26	5.21	0.29	0.34	1.05
0.3% EMS	31.14	30.02	9.09	2.80	1.55	0.09	0.09	0.31
0.4% EMS	42.66	40.15	11.66	1.90	0.79	0.04	0.05	0.16
Overall	27.02	24.63	7.80	4.14	4.22	0.21	0.26	0.69
0.01% SA	7.94	7.27	1.39	0.96	16.00	0.12	0.13	0.69
0.02% SA	12.27	13.33	3.45	1.37	11.50	0.11	0.10	0.40
0.03% SA	21.26	17.98	4.03	1.92	10.83	0.09	0.11	0.47
0.04% SA	32.19	29.33	6.45	1.47	6.13	0.04	0.05	0.23
Overall	18.41	16.97	3.83	1.43	11.11	0.09	0.10	0.45
0.01% HZ	3.99	8.45	2.30	1.97	31.66	0.49	0.23	0.85
0.02% HZ	5.92	17.43	4.90	4.33	36.16	0.75	0.25	0.88
0.03% HZ	18.36	25.63	7.86	7.23	40.16	0.39	0.28	0.92
0.04% HZ	26.60	34.67	10.67	4.15	17.29	0.16	0.12	0.39
Overall	13.72	21.54	6.43	4.42	31.32	0.45	0.22	0.76

& V). The values of mutagenic effectiveness for EMS and SA follow a dose dependent decreasing trend in both varieties. In case of HZ, mutagenic effectiveness exhibited a dose dependent increase but decreased abruptly at the highest dose (0.04%) in both the varieties. The values of effectiveness being as high as 41.44 and 40.16% in var. Avrodhi and var. BG-256, respectively at 0.03% HZ treatment. Based on effectiveness in both the varieties, the order of mutagenic effectiveness was HZ>SA>EMS.

Three criteria *viz*; seedling injury (Mf/I), pollen sterility (Mf/S) and meiotic abnormalities (Mf/Me) were taken into consideration to determine the efficiency of the mutagens. Efficiency varied depending upon the criterion selected for its estimation. Based on seedling injury, the order of efficiency was HZ>EMS>SA; whereas, on the basis of sterility, it was EMS>HZ>SA. Based on the meiotic aberrations induced, EMS was found to be the most efficient mutagen followed by SA and HZ in var. Avrodhi and HZ proved to be the most efficient mutagen followed by EMS and SA in var. BG-256.

DISCUSSION

The frequency and spectrum of chlorophyll mutations are both mutagen and variety dependent in chickpea. For instance, of the mutagens used, EMS induced the highest chlorophyll mutations in both the varieties, followed by HZ and SA. var. Avrodhi, is more sensitive to mutagens than the var. BG-256. Differences between Avrodhi and BG-256 in the chlorophyll mutations induced by mutagens may be due to genotypic differences existing between the two varieties. Both Avrodhi and BG-256 differ phenotypically from each other, the former being a tall and small seeded variety and the later a semi-spreading to erect bold seeded variety. 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 & Bhan, 1977). The maximum induction of xantha mutations in both the varieties suggests that genes for xanthophyll development are readily available for mutagenic action.

Though, EMS induced higher frequency of chlorophyll mutations but the order of mutagenic effectiveness as determined on M_2 plant basis was HZ>SA>EMS. The difference in molar concentrations appear to be the reason for low values of its effectiveness. Contrary to the earlier reports of various workers (Monti, 1968; Prasad, 1972; Nerker, 1977; Sharma & Sharma, 1979), EMS proved to be less effective than HZ and SA in both the varieties. The lower concentrations of EMS and SA and moderate concentrations of HZ proved to be most effective in both the varieties. From breeder's point of view, mutagenic efficiency has more practical value than mutagenic effectiveness because of the fact that an increase in mutation rate cannot be solely achieved at the expense of maximum induced biological damage. Therefore, the values of efficiency provide an idea of extent and type of damage caused by a mutagen in question. Mutagenic efficiency varied depending upon the criterion chosen for its estimation. The mutagenic efficiency decreased with an increase in biological damage in the present study. The most desirable mutagen would be one, which causes less lethality and yields maximum useful mutations. EMS proved to be efficient with regard to sterility and meiotic aberrations. Higher frequency of mutations induced by EMS appears to be the reason for its high efficiency with regard to sterility and meiotic aberrations. On the basis of various criteria selected for mutagenic efficiency, the results have indicated that an efficient mutagen may not be always the effective one. Konzak *et al*. (1965) in barley and Khan and Siddiqui (1992) in mungbean have drawn similar conclusions. It is now well known that mutagenic efficiency and effectiveness depends not only on the type of mutagen and its dose, but also on the genetic architecture of an organism.

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