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Studies on Modification of Mutation Response After Alteration in the Period of Presoaking Treatment of Chilli Seeds

R. SETHUPATHI RAMALINGAM[1]

Studies on the effect of treatments with Ethyl methanesulphonate at two different concentrations on germinating seeds of chillies of different metabolic states have revealed, the occurrence of two sensitive peaks one around 24 to 28 hours and the other around 48 to 56 hours in M_1 generation. In these periods, in M_2 the frequencies of chlorophyll mutations were also relatively high. The chlorophyll mutation spectra induced at different durations, of presoaking varied to a marked degree suggesting that mutation spectra could be altered by varying the presoaking periods.

Although precise control over the induced mutation spectrum is not yet possible, recent studies in wheat, barley and rice indicated that when seeds were treated with chemical mutagens at different times during S-phase of DNA synthesis, the frequency and spectrum of mutation could be altered (Swaminathan and Sarma, 1968, Savin *et al*., 1968, Grant *et al*., 1969; Nair, 1971 and Dekock, 1972). Such studies have not been attempted in chillies (*Capsicum* sp.). The present paper deals with the direct effects readily available in M_1 generation and on M_2 mutation frequency and spectrum in M_2 when the germinating seeds (varied metabolic state) of one cultivar (K. 1) of chillies were subjected to ethyl methanesulphonate.

MATERIAL AND METHODS

The seeds of the K. 1 chilli (*Capsicum annum* L.) were subjected to different presoaking periods ranging from 8 to 60 hours, at intervals of 4 hours. Three hundred and sixty seeds for each treatment were soaked by complete submersion in distilled water for the appropriate periods. In treatments having more than 24 hours of presoaking, seeds were submerged in water for 24 hours and later on kept in covered petridishes lines with moist filter paper for the remaining period of the respective duration to prevent drying and to ensure oxygen availability for the metabolic activity of the germinating seeds. The presoaking was so adjusted, that all the seeds presoaked for different durations became available at one and the same time for treating simultaneously with the chemical mutagen. Untreated dry seeds with moisture content of 12 ÷ 1.0 per cent served as the control. The presoaked seeds were treated with two dose-duration combinations of EMS namely, 50 mM for four hours (Series I) and 75 mM for two hours (Series II), which were prepared afresh with phosphate buffer solution (0.1m) maintained at pH 7. The volume of the solution in

1. Oilseeds Breeder, Agricultural Research Station, Aliyarnagar-642 101.

each treatment was maintained at a proportion ten times that of seeds to facilitate uniform absorption of the mutagens by the seeds. All the treatments were administered at a temperature of 24+2°C. Treated seeds were washed in running water for 30 minutes before sowing.

The M_1 generation was raised in randomised block design with three replications and 120 seeds were sown in each replication adopting uniform spacing (2.5 cm either way) and depth. Data on germination (emergence of cotyledon) survival and height of seedlings (the height was measured from the collar region to tip of the top most leaf) on 30th day and chlorophyll chimeras in M_1 were recorded. The frequency and spectrum of chlorophyll mutations in M_2 were estimated by raising the progenies on M_1 plant and M_1 fruit progeny basis. Statistical comparisons were made by analysis of variance method. Correlation coefficients were worked out between the period of presoaking on one hand and the M_1 effects like reduction in germination, survival and seedling growth on the other. Combinations showing significant correlations were subjected to linear regression analysis.

RESULTS AND DISCUSSION

The germination of seeds and survival of seedlings decreased significantly with the increase in the period of presoaking treatments in both the series (Table I). However, maximum reduction in germination survival was observed at 56 and 48 hours of presoaking in Series

TABLE I: Effect of pre-soaking seeds with EMS on germination, survival, seedling height and occurrence of chlorophyll chimeras in M_1 generation

Mutagen dose and duration of treatment	Period of pre-soaking (hours)	Per cent of control		Seedling height			Per cent M_1 plants showing chlorophyll chimeras
		Germination	Survival	Mean (cm)	± S.E	Per cent of control	
(1)	(2)	(3)	(4)	(5)		(6)	(7)
Control	—	100.0	100.0	20.00	± 0.74	100.0	—
EMS - 50 mM 4 hours (Series I)	8	95.8	71.4	15.50	± 0.73	77.5	—
	12	97.5	75.6	14.99	± 0.89	75.0	0.5
	16	84.1	64.9	16.77	± 0.82	83.9	—
	20	87.9	69.3	13.32	± 0.50	66.6	1.0
	24	70.6	53.3	9.64	± 0.49	48.2	1.3
	28	86.6	66.8	9.32	± 0.63	46.6	—
	32	77.8	64.1	13.02	± 0.76	65.1	1.0
	36	92.5	79.1	14.53	± 0.87	72.7	—
	40	76.7	69.4	15.49	± 0.78	77.5	—

[Contd.

(1)	(2)	(3)	(4)	(5)	(6)	(7)
	44	80.8	52.2	12.97 ± 0.75	64.9	2.0
	48	50.3	31.1	12.34 ± 1.47	61.7	3.4
	52	36.8	22.7	13.11 ± 0.76	65.6	3.1
	56	27.0	20.0	10.03 ± 0.66	50.2	5.3
	60	87.4	68.6	11.86 ± 0.74	59.3	1.0
Difference between periods of pre-soaking		**	**	**		
S.E.		1.6	1.2	1.16		
C.D. (P = 0.05)		4.7	3.5	3.35		
r		0.643*	0.579*	0.413NS		
b		0.54*	0.40*	—		
EMS - 75 mM 2 hours	8	87.3	66.2	15.38 ± 0.81	76.9	—
	12	89.9	70.5	16.04 ± 0.68	80.2	—
	16	89.0	70.0	15.96 ± 0.60	79.8	0.5
	20	89.9	71.1	14.50 ± 0.74	72.5	2.5
	24	78.8	67.3	11.90 ± 0.84	59.5	1.7
	28	98.9	79.2	12.12 ± 0.76	60.6	—
	32	86.6	71.1	10.34 ± 0.52	51.7	—
	36	80.0	66.2	13.38 ± 0.78	66.9	1.1
	40	84.0	67.3	17.74 ± 0.89	88.7	3.1
	44	88.3	63.2	14.40 ± 0.75	72.0	3.9
	48	43.8	17.6	10.22 ± 0.46	51.1	2.0
	52	49.1	20.7	6.92 ± 0.66	34.6	1.8
	56	45.9	21.7	7.74 ± 0.84	38.7	4.0
	60	75.6	65.1	9.40 ± 0.72	47.0	0.7
Difference between periods of pre-soaking		**	**	**		
S.E.		1.7	1.5	1.04		
C.D. (P = 0.05)		4.9	4.3	3.02		
r		0.626*	0.620*	0.666**		
b		0.456*	0.475*	0.406**		

NS, * and ** denote mean values not significant and significant at 5 and 1 per cent levels, respectively.

r and b denote correlation and regression coefficients, respectively.

I and II, respectively. The reduction in height of seedling was significant and was maximum at 28 and 52 hours of presoaking in Series I and II, respectively. The regression analysis showed linear relationship between the periods of presoaking and the reduction in the percentages of germination, survival and seedling height (except in the case of seedling height reduction in Series I). Maximum incidence of chlorophyll chimera was observed in the treatments with 56 hours of presoaking in both the series. Chimeric plants were often met with in the long periods of presoaking (Table I).

The frequencies of chlorophyll mutations observed on M_1 plant, M_1 fruit and M_2 seedling bases were maximum in the treatments with 24 and 44 hours of presoaking in series I and II, respectively (Table II). Low frequencies of mutations were observed in treatments with presoaking durations between 8 and 16 hours. In general, no association could be observed beween the occurrence and type of chlorophyll mutations and the periods of presoaking.

Recent studies on mutagen sensitivity showed that presoaked seeds are more sensitive to mutagens than dry seeds (Savin *et al.*, 1968; Swaminathan *et al.*, 1971; Conger *et al.*, 1973). In the present study, considering germination, survival and seedling height as criteria for sensitivity two peaks could be observed, a minor one around 24 - 28 hours and a major peak around 48 - 56 hours (Table I). In terms of chlorophyll mutation frequency also one peak around 24 hours and the other around 44 hours were observed (Table II). Thus, the time specificity of the sensitive periods was more or less similar irrespective of the dose of EMS and the criteria followed to note the sensitivity.

The fluctuations in sensitivity over different periods have been reported by several workers (Natarajan and Shivashankar, 1965; Savin *et al.*, 1968; Siddic *et al.*, 1970 and Swaminathan *et al.* 1971). Savin *et al.* (1968) reportec two sensitive peaks (at 16 and 28 hours, in barley and attributed causes for such fluctuations also. Natarajan and Shivashankar (1965) reported that the firs DNA synthesis taking place in the cell initials might be the cause for the enhanced sensitivity between 16 and 18 hours of presoaking barley seeds. Autoradiographic studies by Savin *et al.*, (1968) have confirmed this hypothesis. Siddiq *et al.*, (1970) reported sensitive peaks at 11, 18, 22 and 28 hours of presoaking in rice. They explained that the first peak at 11 hours was due to the loss of radioprotective substance, the second peak at 18 hours might coincide the beginning of S phase of DNA replication or the first mitotic division as evidenced from the cytological studies, the first cell division commenced after 22 hours of presoaking and the subsequent peaks probably due to the effect of the mutagen on asynchronously replicating DNA in one chromosome or different chromosomes in a cell.

In the present material (K.1 chillies) the first mitotic division took place after 24 hours of presoaking (Sethupathi Ramalingam, 1976) and the number of dividing cells increased with the duration of presoaking. Osone and Mikaelsen

TABLE II. Frequency of chlorophyll mutations in the M_2 generation of EMS with different periods of presoaking

Mutagen dose and duration of treatment	Period of presoaking with water	Number of plant progenies		Number of M_1 fruit progenies		Number of M_2		Frequency of		
		Scored	Segregated	Scored	Segregated	Seedlings (plants) scored	Chlorophyll mutants	Mutations per 100 M_1		Mutation per 100 M_2 seedlings (plants)
								Plants	Fruits	
Control	—	109	3	228	0	3689	0	—	—	—
EMS - 50 mM 4 hours	8 hours	84	4	159	4	1917	18	4.8	2.5	0.94
	12 ,,	106	18	185	20	2845	44	17.0	10.8	1.55
	16 ,,	89	12	184	13	1706	20	13.5	7.1	1.17
	20 ,,	124	18	169	20	2752	58	14.5	11.8	3.10
	24 ,,	116	34	283	56	4691	175	29.3	19.8	3.73
	28 ,,	104	12	178	13	1811	24	11.5	7.3	1.32
	32 ,,	115	22	210	31	2666	66	20.9	14.8	2.47
	36 ,,	100	14	189	22	2874	55	14.0	11.6	1.91
	40 ,,	146	31	349	42	5014	99	21.2	12.0	1.97
	44 ,,	148	38	308	51	5105	126	25.7	16.6	2.47
	48 ,,	156	35	326	44	5306	108	22.4	13.5	2.04
	52 ,,	124	26	308	37	5039	106	21.0	12.1	2.10
	56 ,,	98	24	292	41	4359	112	24.5	14.0	2.57
	60 ,,	107	20	305	35	4483	98	18.7	11.5	2.19
EMS.75mM 2 hours (series II)	8 hours	90	9	188	9	1668	19	10.0	4.8	1.13
	12 ,,	106	14	246	18	2628	43	13.2	7.3	1.64
	16 ,,	102	11	188	12	1817	39	10.8	6.7	2.15
	20 ,,	104	16	252	24	3972	94	15.4	9.5	2.37
	24 ,,	132	24	224	29	3456	141	16.2	12.9	4.08
	28 ,,	114	29	296	48	4362	204	25.4	16.2	4.65
	32 ,,	109	28	292	37	4449	156	25.7	12.7	3.51
	36 ,,	109	28	238	38	3581	113	25.7	16.0	3.16
	40 ,,	118	31	247	46	4185	226	26.3	18.6	5.40
	44 ,,	120	36	248	51	4398	321	38.0	20.6	6.56
	48 ,,	141	35	302	53	4927	247	24.8	17.6	5.01
	52 ,,	137	28	291	58	4461	187	20.4	14.8	4.19
	56 ,,	123	24	356	49	4674	169	19.5	13.8	3.62
	60 ,,	104	21	334	41	4687	174	20.2	12.3	3.71

(1971) observed that DNA synthesis in the embryo was closely followed by an increase in the mitotic index.

In the present study, the sensitive peak noticed around 24 hours of presoaking might probably be the synchronisation of treatment time with the S-phase of DNA synthesis while the second peak around 44 hours might be attributed to one of the reasons explained by Savin *et al.* (1961) and Siddiq *et al.* (1970) that is, different groups of cells in the meristematic zone initiating the process of cell division at different periods depending upon their position and physiological state.

The spectrum comprised *albina*, *chlorina*, *viridis* and *albo-viridis* types. The spectrum of mutants was influenced by the periods of presoaking. In series I, in treatments with 20, 24, 32, 40, 48 and 56 hours of presoaking, wide spectrum consisting of five types of mutants was generally observed. *Viridis, chlorina and albo-viridis* appeared in most of the periods of presoaking but their frequencies did not bear any relationship with presoaking periods.

In series II, six types of mutants in treatments with 28 hours, five types with 32, 36 and 56 hours, and three types with 8 to 20 hours of presoaking were recorded. *Albina* and *viridis* types were recorded in treatments with all the periods of presoaking. *Viridis* occurred more frequently in treatments with 20, 24, 32 and 40 to 48 hours of presoaking. *Chlorina* type was recorded only in treatments with 24 hours and subsequent periods of presoaking.

There is hardly any precise technique to alter the mutation spectrum in a predictable manner and thereby to achieve directed mutagenesis. The results obtained in the present study showed that besides the increase in frequency of mutation, the spectrum could also be changed in both the series. Differences in the spectrum of mutations due to varying periods of presoaking was evident for the same dose level of EMS. Though *albina* occurred in all the periods of presoaking, the proportion of *albina* was high in all the periods (excepting at 16, 28, 52 and 60 hours) of presoaking in series I and 8 to 16, 28, 36 and 52 hours in series II. Natarajan and Shivashankar (1965) reported an increase in the percentage of *albina* in treatments with EMS after presoaking. *Viridis, chlorina* and *albo-viridis* in series I were encounted in most of the periods of presoaking. Absence of *Xantha* upto 16 hours in series I and *chlorina* upto 12 hours in series I, and upto 20 hours in series II and their occurrence in the prolonged periods of presoaking were the most conspicuous features recorded in the present study. The results of this study suggest the feasibility to alter the relative proportion of kinds of mutations in chillies by administering the mutagenic treatments at different periods of presoaking as reported by Swaminathan and Sharma (1968)in barley, Siddiq *et al.* (1970) in rice and Dekock (1972) in wheat.

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