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ENHANCEMENT OF THE MUTAGENIC EFFECTS OF NITROSO METHYL UREA IN RICE THROUGH ALTERATION IN THE PERIOD OF PRESOAKING OF SEEDS

V. GOPINATHAN NA1R

College of Agriculture, Vellayani, Kerala

Increase in sensitivity of seeds to radiations by presoaking has been demonstrated in different plants. Many investigators have reported that the sensitivity of rice seeds to radiations and chemical mutagens increases with presoaking. However, studies relating to the enhancement of mutation frequency by suitably altering the metabolic condition of the seed by presoaking for various periods have not received much attention in this crop. The present work was undertaken to study the effect of soaking seeds in distilled water for various periods on sensitivity, mutation frequency and spectrum in rice.

Materials and Methods

Mutagenic treatment was given in 3 series ie. with 3 doses of Nitroso methyl urea (NMH) viz. 1 m*M* for 4 hours, 3 m*M* for 2 hours and 3 m*M* for 4 hours. Each series consisted of 11 different periods of presoaking in the range of 8 to 48 hours at intervals of 4 hours. Thus there were 34 treatments including one control. 300 seeds of the variety Co.29, soakad in distilled water were subjected to each treatment. Presoaking in the various treatments commenced at different times and mutagen treatment was done simultaneously. Seeds after treatment were washed in water for one hour and sown immediately.

Survival count and height measurement were made on 30 days old seedlings. Chlorophyll deficient chimeras were recorded at flowering. The first five panicles in order of emergence were labelled in 100 plants selected at random in each of the 22 treatments belonging to the first two series and the control. Materials in the third series were not advanced to the M_2 generation since the number of surviving M_1 plants in certain treatments were very small. The M_2 generation was raised on ear progeny basis. Chlorophyll mutation frequencies were estimated as number of mutations per 100 M_1 ears. The segregation ratio and mutation spectrum were also estimated.

Results and Discussion

The M_1 effects following treatment with NMH after presoaking for different periods are presented in table 1. The magnitude of M, damage differed in the 3 series corresponding to the 3 doses. A high concentration for a short period was more damaging than a low concentration for a long period. The magnitude of

Table 1

M_1 effects in treatment with NMH after different periods of presoaking

Mutagen dose	Period of presoaking of seeds	Seedling survival at 30 days (% of control)	Seedling height at 30 days (% control)	No. of MI plant surviving at flowering	Chlorophyll chimeras No.	Chlorophyll chimeras per 100 M_1 plants
control		100	100	22-	0	0
Series—I						
1 m*M* for 4 hour.	8 hr.	100	98	227	[	0
	12 hr.	100	95	225	1	0.4
	16 hr.	99	92	224	1	0.4
	20 hr.	98	84	224	0	0
	24 hr.	99	84	223	4	1.8
	28 hr.	100	77	226	4	1.8
	32 hr.	99	76	226	I	0.4
	36 hr.	100	78	225	6	2.7
	40 hr.	100	78	227	7	3.1
	44 hr.	98	78	225	6	2.7
	48 hr.	100	79	221	8	3.6
Series—II						
3 *mM* for 2 hour.	8 hr.	98	93	220	0	0
	12 hr.	100	90	227	0	0
	16 hr.	100	90	222	0	0
	20 hr.	100	80	214	1	0.5
	24 hr.	97	66	208	3	1.4
	28 hr.	94	54	184	4	2.2
	32 hr.	92	52	169	7	4.2
	36 hr.	88	56	177	7	4.0
	40 hr.	89	59	18	5	2.6
	44 hr.	g9	62	185	1	0.5
	4S hr.	89	64	196	4	2,0
Series— III						
3 *mM* for 4 hour.	8 hr.	;00	92	227	2	0.9
	12 hr.	98	90	225	0	0
	16 hr.	92	84	210	6	2.9
	20 hr.	6 ·	66	136	6	4.4-
	24 hr.	23	48	40	0	0
	2S hr.	7	33	11	0	0
	32 hr.	5	27	7	0	0
	36 hr.	6	27	12	0	0
	40 hr.	14	29	26	3	11.5
	44 hr.	19	33	27	3	11.1
	48 hr.	26	39	41	3	0.7

damage was maximum at the highest dose and greater in terms of lethality (survival reduction) than injury (height reduction).

In each series the percentages of survival and seedling height decreased, ie. sensitivity increased, with increasing periods of presoaking. Sensitivity was reported to increase by presoaking seeds in water before treatment with chemical mutagens by Swaminathan *et al.* (1970) in rice, Brunner *et al.* (1968) in barley and Robbelen (1965) in *Arabidopsis*. The increase in sensitivity by presoaking was attributed to the leaching of endogenous protective substances, changes in the metabolic condition of the cells and DNA synthesis.

Sensitivity reached a maximum at 32 hours presoaking. There was a decrease in sensitivity when presoaking was extended beyond this period. The time specificity of the sensitivity peak was the same irrespective of the dose of the mutagen and the criterion employed to estimate damage. In dehulled seeds of rice, Swaminathan *et al.* (1970) found a drastic increase in sensitivity to ethyl methane sulphonate after presoaking for 18 to 22 hours. Similarly, peak sensitivity to mutagen treatment was obtained in barley seeds presoaked for 16 hours (Savin *et al.* 1968). in maize seeds presoaked for 26 hours (Latteral, 1961) and in *Arabidopsis* seeds presoaked for 12to 15 hours (Robbelen, 1965). Natarajan and Shivasanker (1965) postulated that the first DNA synthesis taking place in the celt nitials was the most important factor responsible for the increased sensitivity during the 16 to 18 hour presoaking period in barley seeds Autoradiographic studies by Savin *et al.* (1968) have confirmed this hypothesis. In rice, autoradiographic studies by Ayengar *et al.* (1969) have revealed that DNA synthesis is initiated between 24 and 32 hours in seeds with hull and between 12 and 16 hours in seeds without hull. Thus the peak period of sensitivity to NMH observed in the present study corresponds to the time of DNA synthesis in the initial cells. It therefore appears that the first DNA synthesis taking place in the ceil initials is the most important factor responsible for the peak sensitivity at the 32 hours presoaking period.

The frequencies of chlorophyll mutations estimated on M_1 ear basis and the mean segregation ratio are presented in table 2. The frequencies increased with increase in presoaking time reaching a maximum at 40 to 44 hours. The mean segregation ratio also increased with increase in the period of presoaking. Conspicuous increases in frequency were obtained during the periods 16 to 20 hours and 24 to 28 hours. This enhanced efficiency during the 16 to 28 hour period might be attributed to the synchronisation of treatment time with 'S' phase of DNA synthesis. Ayengar *et al.* (1969) also observed a sharp rise in mutation frequency during the 24 to 32 hour period and a further increase beyond 32 hours reaching a maximum at 48 hours. The sudden increase in mutation frequency in seeds observed by Ismail (1969) and Siddiq *et al.* (1970) also correspond to the period of DNA synthesis. Natarajan and Shivasankar (1965), Savin *et al.* (1968)

Table 2

Mutagenic effects in the M_2 generation (chlorophyll mutations) in treatment with NMH after different periods of pre-soaking

Mutagen dose	Period of pre-soaking of seeds	Number of M_1 ear progenies scored	Number of M_1 ear progenies Segregating	Mutation frequency (per 100 M_1 ears)	% of mutants in segregating progenies, segregation ratio)
Control		421	0		
Series—I					
1 m*M* for 4 hours.	8 hr.	445	3	0.7	6.5
	12 hr.	455	7	1.5	7.4
	16 hr.	447	9	2.0	8.2
	20 hr.	410	33.	8.0	11.5
	24 hr.	436	37	8.5	10.9
	28 hr.	428	23	5.4	13.5
	32 hr.	402	28	7.0	11.5
	36 hr.	377	29	7.7	15.6
	40 hr.	428	36	8.4	13.1
	44 hr.	348	43	12.4	17.7
	48 hr.	426	38	8.9	13.5
Series— II					
3 mM for *2* hours.	8 hr.	394	12	3.0	12.4
	12 hr.	392	19	4.8	11.6
	16 hr.	413	23	5.6	10.2
	20 hr.	415	13	3.1	8.0
	24 hr.	394	25	6.3	11.7
	28 hr.	391	46	11.8	13.2
	32 hr.	437	37	8.5	10.8
	36 hr.	430	58	13.5	16.8
	40 hr.	412	76	18.4	15.8
	44 hr.	379	59	15.6	16.1
	48 hr.	391	51	13.0	16.2

and Mikaelsen (1969) in barley and Robbelen (1965) in *Arabidopsis* have also observed that mutation frequency was the highest when mutaginic treatment was done at the time of DNA synthesis.

The mutation spectrum presented in table 3 indicate that the relative percentage of different types of mutations were influenced by the period of presoaking. *Albina, viridis* and *chlorina* mutants were present at all the stages of presoaking

Table 3

Spectrum (Relative %) of chlorophyll mutations in the M_2 generation in treatment with NMH after different periods of presoaking

Mutagen dose	Period of presoaking of seeds	Total No. of mutations	Spectrum (Relative %) of chlorophyll mutations							
			A	X	V	C	AV	S	T	Others
Series—I										
1 m*M* for 4 hours.	8 hr.	3			67	33				
	12 hr.	7	29			42		29		..
	16 hr.	9	11		33	34	11	11		..
	20 hr.	33	21	6	25	15	18	9	3	3
	24 hr.	37	19	11	38	11	8	8		5
	28 hr.	23	35		22	22	17	.		4
	32 hr.	28	35	7	29	21	4		4	..
	36 hr.	29	34	3	15	18	10	3	10	7
	40 hr.	36	25		30	25	3	6	3	8
	44 hr.	43	42	4	12	12	16		7	7
	48 hr.	38	44	3	3	24	13	3	10	..
Series—II										
3 m*M* for 2 hours.	8 hr.	12	16		34	26	16	..		8
	12 hr.	19	16		32	10	21	5		16
	16 hr.	23	22		17	17	26	9		9
	20 hr.	13	23		31	31		15		..
	24 hr.	25	16	4	32	16	24		8	
	28 hr.	46	19	2	38	2	22			17
	32 hr.	37	33		13	13	11	3	X	19
	36 hr.	58	35	3	28	17	3	7		7
	40 hr.	76	38	13	17	14	4			14
	44 hr.	59	48	7	24	10	5	3	3	
	48 hr.	51	26	17	27	8	8	2		12

and predominated the chlorophyll mutation spectrum. The relative percentage of *albina* increased with the length of presoaking whereas the frequencies of *viridis* and *chlorina* were not considerably altered. *Xantha* and *tigrina* were very rare and appeared from 20 hours presoaking onwards. The increase in the relative percentage of *albina* with increasing period of presoaking and the late appearance of *xantha* and *tigrina* were the characteristic features of the mutation spectrum. An increase in the percentage of *albina* following treatment of bariey seeds with ethyl methane sulphonate after presoaking was reported by Natarajan and

Shivasankar (1965). In 'S' phase fractionation experiments in barley, Swaminathan (1969) has reported a delayed appearance of *xantha* and *tigrina* mutants. The time specificity observed for the appearance of these rare mutations might be because of the relatively lesser number of loci concerned in their determination.

Changing the spectrum of mutations in a predicatable manner and thereby achieving directed mutagenesis is an important goal of current mutation research. Grant and Heslot (1966) reported that different chromosome regions exhibited cyclic changes in duplication and in sensitivity to mutagens and suggested that if cells were subjected to pulse treatment with a mutagen specifically affecting the chromosome under duplication, it could be expected that particular sites of chromosomes would be affected and specific mutations would be induced. Swaminathan (1969) concluded that since DNA replication along a chromosome is asynchronous in time sequence, it would be possible to affect groups of loci preferentially by administering the treatment for short periods at different stages of the 'S' phase. Thus the relative frequency of different types of mutations could be manipulated by synchronising the treatment with the time of replication of a specific locus. The alteration of mutation spectrum observed in the present investigation indicate scope for further efforts in this line towards attaining specificity of mutations in rice.

Summary

The effect of presoaking seeds on sensitivity and mutation frequency in rice was studied. Treatments were given in 3 series ie. with 3 doses of NMH Each series consisted of 11 different periods of presoaking in range of 8 to 48 hours. Mutagenic effects were estimated in the M_1 and M_2 generations and interpreted.

Sensitivity to NMH increased with the length of presoaking and reached a maximum at 32 hours. There was a decrease in sensitivity when presoaking was extended beyond this period. The time specificity of the sensitivity peak was independant of the dose of mutagen and the criteria adopted for its estimation. The peak period of sensitivity corresponds to the time of DNA synthesis in the initial cells It appears that the first DNA synthesis in the initial cells is responsible for the peak sensitivity at 32 hours presoaking.

The frequency of chlorophyll mutations increased with the length of presoaking reaching a maximum at 40 to 44 hours. Conspicuous increase were obtained during the periods 16 to 20 hours and 24 to 28 hours. The enhanced efficiency during the 16 to 28 hour period can be attributed to the synchronisation of treatment time with the 'S' phase of DNA synthesis. The spectrum of mutations indicated predominance of *albina, viridis* and *chlorina*. *Xantha* and *tigrina* were very rare and appeared only from 20 hours onwards. The increase in relative per cent of *albina* with the length of presoaking and the late

appearance of *Xantha* and *tigrina* were the characteristic features of the mutation spectrum. The change in spectrum with different periods of presoaking indicate scope for further efforts towards attaining mutation specificity.

സംഗ്രഹം

8 മുതൽ 48 മണിക്കൂർവരെയുള്ള വിവിധകാലയളവുകളിൽ കുതിർത്ത നെൽവിത്തു് NMH എന്ന രാസഉൽപരിവർത്തക പ്രയോഗത്തിനു് വിധേയമാക്കി. 32 മണിക്കൂർ കുതിർത്ത വിത്തിനു് സംവേദകത്വം ഏറ്റവും കൂടുതലായികണ്ടു. സംവേദകത്വത്തിന്റെ ഈ ഉത്പതമകാലത്തിra" നിദാനം പ്രാരംഭികകോശങ്ങളിൽ ഈ സമയത്തു നടക്കുന്ന DNA സംശ്ലേഷണമാണെന്നു് അനുമാനിയ്ക്കാം. 16 മുതൽ 28 മണിക്കൂർവരെ കുതിർത്ത വിത്തുകളിൽ ക്ലോറോഫിൽ ഉൽപരിവർത്തന ആവൃത്തി അധികരിച്ചതായി കണ്ടു. രാസഉൽപരിവർത്തകപ്രവർത്തനം DNA സംശ്ലേഷണത്തിന്റെ ആരംഭദശയുമായി യോജിച്ചു വന്നതായിരിക്കാം ഇതിനുകാരണം.

ഉൽപരിവർത്തകപ്രവർത്തനത്തിനു fija3a_jgg കുതിക്കൽ കാലാവധി കൂടുന്നതനുസരിച്ചു് *allina* യുടെ ആപേക്ഷിക ശതമാനം കൂടുന്നതായി കണ്ടു. *Xantha, tigrina* എന്നീ ഉൽപരിവർത്തനങ്ങളും വളരെ കുറഞ്ഞ raTOgojito? 20 മണിക്കൂറിനുശേഷമുള്ള കാലയളവിൽ മാത്രം പ്രത്യക്ഷപ്പെട്ടു. കുതിക്കൽ കാലയളവിലുള്ള വ്യത്യാസമനുസരിച്ചു് ഉൽപരിവർത്തിതങ്ങളുടെ തരത്തിലും എണ്ണത്തിലുമുള്ള വ്യത്യാസം ഉൽപരിവർത്തനനിർദ്ദിഷ്ടത എന്ന ലക്ഷ്യത്തിലേക്കു് വഴിതെളിക്കുമെന്നു് പ്രത്യാശിക്കാം.

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