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EFFECT OF COMBINED MUTAGENIC TREATMENTS ON SENSITIVITY AND MUTATION FREQUENCY IN RICE

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Combination of mutagenic treatments is one of the methods employed for the enhancement of mutagenic efficiency. This approach makes use of the fact that various physical and chemical mutagens induce different spectra of mutations. Thus, combined treatments with radiations and chemical mutagens will increase the mutation frequency and enlarge the mutation spectrum. The present study was undertaken to examine the possibility of enhancing the induction of mutations in rice through combined mutagenic treatments.

Materials and Methods

Seeds of the rice variety C 0.29 were subjected to two sets of combination treatments with radiations (fast neutrons and gamma rays) and nitrosomethyl urea (NMH).

i) Fast neutrons + NMH:— Three doses of fast neutrons, viz. 705, 968, and 1170 rad and four doses of NMH, viz. 0.97, 1.94, 2.91 and 3.88 mM were employed in all the 12 possible combinations. Neutron irradiation was done with the Standard Neutron Irradiation Facility at the ASTRA reactor of the International Atomic Energy Agency, Vienna.

ii) Gamm rays + NMH:— Three doses of gamma rays viz. 10, 20 and 30 krad and two doses of NMH, viz. 0.48 and 0.97 mM were employed in all the 6 possible combinations. Gamma irradiation was done by using the gamma cell at the I. A. R. I., New Delhi.

Irradiated seeds were soaked in distilled water for 16 hours and treated with NMH by keeping immersed in aqueous solution of the mutagen for eight hours. They were sown in three replications. Survival counts and height measurements of seedlings were made on the 30th day. The M_2 generation was raised on M_1 ear progeny basis. Chlorophyll mutations were scored on M_1 ear and M_2 plant basis and frequencies calculated. The segregation ratio of mutants was estimated in each dose combination as the percentage of mutants in mutated ears.

Results and Discussion

The data on survival and seedling height in the M_1 generation, chlorophyll mutation frequencies estimated as number of mutations per 100 M_1 ears and as number of mutants per 100 M_2 plants, and segregation ratio of mutants in the M_2 generation are presented in Table 1. The M_1 and M_2 data

Table 1

M, effects and M_2 chlorophyll mutations in combination treatments of radiations and NMH.

Mutagen		M_1 effects (% of control)		chlorophyll mutations		
Radiation dose (rad)	NMH dose (mM)	Seedling survival (30th day)	Seedling height (30th day)	Mutations per 100 M_1 ears	Mutants per 100 M_2 plants	Segregation ratio (%)
I. Fast neutrons + NMH						
Control		100	100	0	0	0
705		100	95	9.5	0.96	11.0
968	—	98	93	9.9	1.05	10.4
1170	—	98	92	11.1	1.07	8.5
	0.97	96	90	9.2	0.92	13.9
	1.94	80	80	10.6	1.31	13.6
	2.91	60	72	12.7	1.34	12.6
—	3.88	48	69	14.0	1.50	13.0
705	0.97	93	85	8.6	0.87	12.6
,,	1.94	71	78	13.6	2.37	19.8
,,	2.9i	54	69	17.5	3.57	20.7
,,	3.88	43	64	26.0	3.91	17.4
968	0.97	91	78	34.2	2.35	16.4
,,	1.94	64	74	17.4	3.57	19.2
,,	2.91	61	67	19.5	3.15	18.3
	3.88	45	62	18.8	3.23	19.6
1170	0.97	94	77	11.4	1.22	12.6
,,	1.94	61	70	18.0	2.94	16.0
,,	2.91	53	66	18.3	3.06	18.9
,,	3.88	44	6!	26.0	3.94	16 0
II. Gamma rays	NMH					
Control		100	100	0	0	0
10.000	—	98	92	6.2	0.64	11 0
20,000		99	88	7.3	1.30	10.2
30,000	—	99	83	9.2	1.31	11.8
	0.48	94	88	5.8	0.52	8.5
—	0.97	91	83	8.8	0 90	12.8
10,000	0.48	94	84	2.8	0.35	16.5
	0.97	90	79	3.9	0.62	16.5
20,000	0.48	90	80	5.7	0.34	4.6
	0.97	88	78	7.5	3.53	38.3
30 000	0.48	91	77	8.2	1.54	13.2
	0.97	86	70	12.5	2.87	18.9

are tabulated in a two way table to assess the interaction effects of the two mutagens in the combined treatments and presented in Table 2. Expected values estimated for the combined treatments on the basis of additive effects are also given in this table.

The percentages of survival and seedling height in either of the combination treatments did not differ considerably from values expected on the basis of additive effects (Table 2-i & ii). Soriano (1968) found that ethyl methane sulphonate when applied after irradiation of rice seeds with fast neutrons did not affect seedling height and fertility. Prasad (1968) observed that NMH in combination with gamma rays acted effectively in wheat, while such synergism was not observed in the case of gamma rays and ethyl methane sulphonate.

Chlorophyll mutation frequencies in the M_2 generation of combination treatments estimated as number of mutations per 100 M_1 ears were not higher than those expected from simple additive effects of the mutagens (Table 2-iii). But the frequency when estimated as number of mutants per 100 M_2 plants revealed a different picture. The mutant frequencies were higher in combination treatments than the sum of frequencies in single treatments. Such positive synergistic effect of mutagens on mutant frequencies was more pronounced at combinations of higher doses of fast neutrons and NMH (table 2-iv). Thus the higher dose combinations of fast neutrons and NMH were more effective than single treatments in respect of mutant frequency. Aastveit (1968) and Sharma and Swaminathan (1970) in combination treatments of gramma rays and EMS in barley observed similar synergistic effects of mutagens on mutant frequencies.

The percentages of mutants in mutated ears (segregation ratio) in combination treatments were higher than those in single treatments (table 2-v). The ratios at the higher dose combinations were considerably high indicating a strong positive synergistic effect. This high segregation ratio was responsible for the higher mutant frequencies in combinations of higher doses of fast neutrons and NMH. The increase in segregation ratio reflects a corresponding increase in the size of the mutated sector.

The synergistic effects in combination treatments of mutagens are explained in several ways. Scarascia - Mugnozza (1969) has stated that if one mutagen sensitises previously protected sites to the second mutagen, more than additive effects can be expected from sequential application of mutagens. According to Sharma and Swaminathan (1970), inactivation of repair enzymes by the second mutagen will increase the chances of potential mutational lesions induced by the first mutagen to get fixed. Bhatia (1970) has stated that radiations induce changes in the properties of biological membranes and this will enhance the penetration of the chemical mutagen when it is given after irradiation, leading to synergistic effects.

Table 2

Interaction effects of radiations and NMH in combination treatments in relation to M_1 damage and M_2 chlorophyll mutations

Radiation doses (rad)	NMH doses (mM)					
	0	0.48	0.97	1.94	2.91	3.88
1	2	3	4	5	6	7
(i) Survival of seedlings -30th day (% of control)						
Fast neutrons						
Control	100	—	96	80	60	48
705	100	—	93 (96)	71 (80)	54 (60)	43 (48)
968	98	—	91 (93)	64 (78)	61 (59)	45 (47)
1170	98	—	94 (93)	61 (78)	53 (59)	44 (47)
Gamma rays						
Control	100	94	91	—		—
10,000	98	94 (92)	90 (89)			
20,000	99	90 (93)	88 (90)	—	—	—
30,000	99	91 (93)	86 (90)		—	—
(ii) Height of seedlings - 30th day (% of control)						
Fast neutrons						
Control	100	—	90	80	72	69
705	95	—	85 (86)	78 (7(0	69 (68)	64 (66)
968	93	—	78 (84)	74 (74)	67 (67)	62 (64)
1170	02		77 (83)	70 (74)	66 (66)	61 (64)
Camilla rays						
Control	100	88	83		—	—
10.000	92	84 (81)	79 (76)			—
20,000	88	SO (77)	78 (73)			—
30,000	83	77 (73)	70 (69)	—		

Table 2 (Continued)

1	2	3	4	5	6	7
(iii) Mutations per **100** M_1 **ears**						
Fast neutrons						
Control	0		9.2	10.6	12.7	14.0
705	9.5		8.6 (18.7)	13.6 (20.1)	17.5 (22.2)	26.0 (23.5)
968	9.9	—	14.2 (19.1)	17.4 (20 5)	19.5 (22.6)	18.8 (23.9)
1170	11.1	..	11.4 (20.3)	18.0 (21 7)	18.3 (23.8)	26.0 (25.1)
Gamma rays						
Control	0	5.8	8.8	—	—	—
10,000	6.2	2.8 (120)	3.9 (15.0)	—		
20,000	7.3	5.7 (13.1)	7.5 (16.1)			—
30,000	9.2	8.2 (15.0)	12.5 (18.0)	—		
(iv) Mutants per **100** M3 plants						
Fast neutrons						
Control	0		0.92	1.31	1.34	1.50
705	0.96	—	0.87 (1.88)	2.37 (2.27)	3.57 (2.30)	3.91 (2.46)
968	1.05	—·	2.35 (1.97)	3.57 (2.36)	3.15 (2.39)	3.23 (2.55)
1170	1.07		1.22 (1.99)	2.94 (2.38)	3.06 (2.41)	3.94 (2.57)
Camilla rays						
Control	0	0.52	0.90			...-,
10,000	0.64	0.35 (1.16)	0.62 (1.54)	—	=	—
20,000	1.30	0.34 (1.82)	3.53 (2.20)	— —	— —	
30,000	1.31	1.54 (1.83)	2.87 (2.21)	—	-	=
.(v) Percentage of mutants in mutated ears (segregation ratio)						
Fast neutrons						
Control	0		13.9	13.6	12.6	13.0
705	11.0		126	19.8	20.7	17.4
968	10.4		16.4	19.2	18.3	19.6
1170	8.5		12.6	16.0	18.9	16.0

Table 2 (Continued)

1	2	3	4	5	6	7
Gamma rays	0	8.5	12.8			—
10,000	11.0	16.5	16.5			—
20.POO	10.2	4.6	38.3			—
30,000	11.8	13.2	18.9			—

Expected values For the combined treatments on the basis of additive effects are given in brackets below the observed values.

Summary

Puce seeds were subjected to two sets of combination treatments of radiations and NMH. The effects of mutagenic treatments in the M_1 and M_2 generations were recorded and discussed. Mutation frequencies estimated as number of mutations per 100 M_1 ears were not higher than the values expected on the basis of additive effects. When estimated as number of mutants per]00 M plants, the frequencies revealed more than additive effects. The synergistic effect on mutant frequencies was due to increase in the segregation ratio of mutants. This effect was more pronounced at the higher dose combinations of fast neutrons and NMH.

സംഗ്രഹം

വികിരണത്തിനും രാസഉൽപരിവർത്തകപ്രയോഗത്തിനും തുടർച്ചയായി വിധേയമാക്കുന്നതു വഴി ഉൽപരിവർത്തിതങ്ങളുടെ എണ്ണത്തിലും തരത്തിലും സാരമായ വ്യതിയാനം ഉണ്ടാകുന്നതായി പരീക്ഷണങ്ങൾ തെളിയിച്ചിട്ടുണ്ട്. നെൽവിത്തിനെ വികിരണത്തിനും (ഗാമാ, ന്യൂട്രോൺ) NMH എന്ന roonx>gro?><xj<o1oj&<OTJ)ifl><wj>1rrao തുടർച്ചയായി വിധേയമാക്കുകയും അവയുടെ പ്രവർത്തനം മൂലം ഉളവായ ഉൽപരിവർത്തിതങ്ങളെ രണ്ടാം തലമുറയിൽ പഠിക്കുകയും ചെയ്തു. തുടർച്ചയായ ഉൽപരിവർത്തകപ്രയോഗം മൂലം പർണ്ണഹരിതശൂന്യ ഉൽപരിവർത്തിതങ്ങളുടെ എണ്ണം സാരമായി വർദ്ധിച്ചു. ഈ വർദ്ധനവ് ഉൽപരിവർത്തകങ്ങളുടെ കൂട്ടായ പ്രവർത്തനത്തിന്റെ ഫലമാണെന്ന് അനുമാനിക്കാം.

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