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EFFICIENT MUTAGENESIS IN COWPEA*

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Cowpea is widely grown in India as a highly esteemed grain legume crop. Being a self-pollinated diploid species, it is ideally suited for genetic improvement through mutation breeding. The present investigation was undertaken to study the effects of gamma ray and ethyl methanesulphonate (EMS) treatments on cowpea in M_1 and M_2 generations and to identify the most effective and efficient doses of the two mutagens,

Materials and Methods

The biological material was seeds of New Era variety of cowpea. Seeds containing 12% moisture were exposed to gamma rays at doses of 5, 10, 15, 20, 25 and 30 krad. Prior to ethyl methanesulphonate treatment, seeds were soaked for 2 hours in water. The treatment was done by immersing pre-soaked seeds for 6 hours **in** ethyl methanesulphonate solutions at concentrations of 0.1, 0.2, 0.3, 0.4, 0,5, 0.6 and 0.7 per cent with intermittent shaking. During the treatment process, the temperature of the solutions was maintained at 27 $\pm$ 1°C.

Treated seeds were sown in three replications along with untreated control. Observations on seed germination, survival, plant height and pollen fertility were made in the M_1 generation. Plant survival counts and height measurements were made on the 30th day of sowing. Pollen fertility was studied by staining pollen grains extracted from mature flower buds in 1:1 acetocarmine glycerine solution. Well stained and properly filled pollen grains were scored as fertile and others as sterile. Lethality, injury and sterility were estimated on the basis of reduction in plant survival, plant height and pollen fertility respectively, in comparison with the control.

The M_2 generation was grown as M_1 plant progenies. The progenies segregating for chlorophyll mutations were scored to calculate mutation frequency per 100 M_1 plants. Normal seedlings and chlorophyll mutants were counted to calculate mutant frequency per 100 M_2 plants, in segregating progenies, normal seedlings and mutants were counted and segregation percentage of mutants calculated. Mutagenic effectiveness and efficiency were estimated using the formulae suggested by Konzak *et at.* (1965).

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Results and Discussion

Mutagenic effects in M_1 generation

The effects of the mutagens in M_1 generation are presented in Table 1.

Germination of seeds was not affected by gamma rays even at the highest dose employed, while a progressive decrease in germination with increasing doses of ethyl methanesulphonate was observed. Germination reduction following ethyl methanesulphonate treatment was reported by Narsinghani and Kumar (1970).

Survival of plants, estimated on the basis of number of seeds sown, was reduced by both mutagens. Ethyl methanesulphonate induced lethality was due to inhibition of germination, whereas gamma irradiation induced lethality was due to post-germination on mortality.

Plant height was reduced by gamma irradiation as well as ethyl methanesulphonate treatment. The observation is in accordance with the findings of Wellensiek (1965) in pea and Constantin *et al.* (1976) in soybean. Ethyl methanesulphonate was found to cause more severe growth reduction compared to gamma rays.

Pollen fertility was found to be decreasing with increasing doses of both mutagens. Fertility reduction in pea following gamma irradiation and ethyl methanesulphonate treatment was reported by Wellensiek (1965). Gaul *et al.* (1966) attributed radiation induced sterility to chromosome abberations and chemical induced sterility to cryptic deficiencies and specific gene mutations.

Mutagenic effects in M_2 generation

The data relating to the mutagenic effects in M_2 generation are presented in Table 2.

Mutation frequency estimated as the number of mutations per 100 M_1 plants increased with increasing doses of gamma rays as well as methanesulphonate. On M_2 plant basis, similar dose dependence of frequency was observed only for gamma rays.

In gamma ray treatment series, segregation percentage was maximum (8.41) at 30 krad treatment and with ethyl methanesulphonate, 0.4 per cent treatment gave the highest (12.82) segregation percentage. High segregation percentages facilitate easy selection of mutants since they relate to high frequencies of mutants in segregating populations.

Mutagenic effectiveness and efficiency

Konzak *et al.* (1965) proposed the terms 'effectiveness' as a measure of mutations in relation to dose and 'efficiency' as an estimation of mutation rate in

Table 1

Effects of mutagens in M_1 generation

Mutagen and dose	Percentage over control				M_2 damage (percentage)		
	Germination	Survival	Plant height	Pollen fertility	Lethality	Injury	Sterility
Gamma ray (krad)							
5	100.00	98.58	78.81	98,22	1.42	21.11	1.78
10	100.00	97.89	79.48	95.96	2.11	20.52	4.04
15	100.00	91.55	79.71	89.01	8.45	20.29	10.99
20	100.00	91.55	70.27	79.31	8.45	29.73	20,69
25	100.00	92.25	72.28	73.74	7.75	27.72	26.26
30	100.00	93.30	79.98	71.50	6.70	20.02	28.50
EMS (%)							
0.1	100.00	96.90	80.66	98.96	3.10	19.34	1.04
0,2	96.67	95.03	74.20	98.37	4.97	25.80	1.63
0.3	93.33	93.62	62.95	98.09	6.38	37.05	1.91
0.4	90.00	87.23	67.33	98.06	12,77	32.67	1.94
0.5	86.67	85.82	65.80	97,27	14.18	34.20	2.73
0.6	76.67	79,43	52.57	90.57	20.57	47.43	9.43
0.7	46.67	44.68	39.29	89.54	55.32	60.71	10.46

Table 2

Mutagenic effects in M_2 generation (chlorophyll mutations)

Mutagen and dose	Mutation frequeucy		Segrega-tion %	Mutagenic effective-ness M X100 / tc or krad	Mutagenic efficiency		
	Per 100 M_1 plants	Per 100 M_2 plants			M X 100 / L	M X 100 / I	M X 100 / S
Gamma ray (krad)							
5	3.80	0.30	8.04	76.00	267.61	17.93	213.48
10	10.00	0.42	3.63	100.00	473.93	48.73	247.52
15	15.19	1.01	6.35	101.27	179.76	74.86	138.22
20	19.28	1.04	5.49	96.40	228.17	64.85	93.19
25	20.51	1.38	5.70	82.04	264.65	73.99	78.10
30	25,00	2.29	8.41	83.33	373.13	124.88	87.72
EMS (%)							
0.1	1.25	0.07	9.09	208.33	40.32	6.46	120.19
0.2	2.35	0.15	4.17	195.83	47.28	9.11	144.17
0.3	3.23	0.17	8.33	179.44	50.63	8.72	169.11
0.4	8.79	1.15	12.82	366.25	68.83	26.91	453.09
0.5	8.96	0.95	12,20	298.67	63.19	26.20	328.21
0.6	9.21	0,82	10.11	255.83	44.77	19.42	97.67
0.7	12.50	1.37	10.43	297.92	22.60	20.59	119.53

M = Mutation frequency on M1 plant basis

tc = Time of treatment x concentration of EMS

L -Lethality; I = Injury; S = Sterility

relation to M_1 damages such as lethality, injury and sterility. Chlorophyll mutations are widely employed in assessing mutagenic effectiveness and efficiency on the assumption that other types of mutations are induced with frequencies parallel to that of chlorophyll mutations (Kawai, 1969). The effectiveness and efficiency of the mutagens in inducing chlorophyll mutations are estimated and presented in Table 2. Effectiveness of gamma rays was found to increase upto 15 krad dose, there was a reduction at still higher doses. Among the gamma ray doses employed, 10 krad was the most efficient when efficiency was estimated on the basis lethality or sterility, while 30 krad proved to be the most efficient dose on injury basis. With regard to ethyl methanesulphonate, 0.4 per cent treatment was the most effective as well as efficient irrespective of whether the criterion adopted for the estimation of efficiency was lethality, injury or sterility.

Summary

Studies were undertaken to obtain precise information on the effects of six doses of gamma rays (5 to 30 krad) and seven concentrations of ethyl methanesulphonate (0.1 to 0.7 per cent) on cowpea.

Gamma ray and ethyl methanesulphonate treatments resulted in physiological damages in M_1 generation as evidenced by the reduction in survival of plants, plant growth and fertility. Mutation frequency estimated on M_1 plant basis increased with increasing doses of gamma rays and ethyl methanesulphonate. Effectiveness of gamma rays increased with increasing doses upto 15 krad and there was a reduction at still higher doses. Most efficient gamma ray dose was found to be 10 krad when efficiency was estimated on the basis of lethality or sterility, while on injury basis 30 krad was the most efficient dose. Among the different doses of ethyl methanesulphonate tried, 0.4 per cent was the most effective as well as efficient, irrespective of the criterion adopted for the estimation of efficiency.

സംഗ്രഹം

ഗാമാരശ്മിയും ഈതൈൽ മീതെയിൻസൾഫൊണേറും ഉപയോഗിച്ചു നൃ ഇറ എന്നയിനം വൻപയറിൽ ആകസ്മികവ്യതിയാനം കൈവരുത്തുന്നത് സംബന്ധിച്ചു പഠനങ്ങൾ നടത്തുകയുണ്ടായി. എം1 ചെടികളുടെ എണ്ണം അടിസ്ഥാനമാക്കി ഉൽപരിവർത്തന ആവൃതി കണക്കാക്കുമ്പോൾ, ഉൽപരിവർത്തക തോത് കൂടുന്നതനുസരിച്ച് ആവൃതി കൂടുന്നതായി കാണപ്പെട്ടു. ഗാമാവികിരണ തോതുകളിൽ ഏറ്റവും ഉൽപരിവർത്തകക്ഷമമായത് അതിജീവനക്കുറവോ പരാഗവന്ധ്യതയോ അടിസ്ഥാനമാക്കി കണക്കാക്കുമ്പോൾ 10 കിലോറാഡും സസ്യക്ഷതംഭവിച്ച അടിസ്ഥാനമാക്കി കണക്കാക്കുമ്പോൾ 30 കിലോറാഡും ആണെന്നു തെളിഞ്ഞു. ഈതൈൽ ffIleKincfflIfii സൾഫൊണേറു തോതുകളിൽ 0.4 ശതമാന പ്രയോഗമാണ് ഏറ്റവും ഉൽപരിവർത്തകക്ഷമമായതെന്നും കണ്ടു.

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