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Effectiveness and Efficiency of Single and Combined Treatments of Physical and Chemical Mutagens in Grasspea (*Lathyrus sativus* L.)

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Abstract: Mutagenic effectiveness and efficiency of γ-rays, EMS, NG and their combinations were studied in grasspea, cv. Dhenkanal Local. NG was 40 times effective than EMS and 1000 times effective than γ-rays. Combination treatments were exceptionally more effective than single treatments. All the M_1 parameters except seedling root growth showed varied response with doses and mutagens used. Hence, root length can serve as a reliable criterion for estimation of mutagen efficiency at even seedling stage. Among mutagens, NG was more efficient and EMS and γ-ray were more or less equiefficient,

Key words: Mutagenic effectiveness and efficiency · Gamma-rays · Ethyl methanesulphonate (EMS) · N-ethyl-N-nitro-N-nitrosoguanidine (NG) · *Lathyrus sativus* L.

INTRODUCTION

Despite high protein content (21.8-40%) and high abiotic stress resistance, *Lathyrus sativus* L. is still remains slow runner in productivity owing to undersirable linkage of component traits including BOAA content with seed yield [1]. Besides, it harbours high neurotoxin (β-N-oxalyl amino alanine) content in seed and leaf. Turning off or elimination of one or more genes involved in biosynthesis of neurotoxin and breaking of the aforesaid linkage drag besides exploring new gene(s) or gene complexes for improved yield components following mutagenesis could be a practicable proposition. Tripathy and Lenka [2] could able to isolate few high yielding mutants with neurotoxin content less than 0.1% using NG. One of the important criteria in mutation research is to produce desirable changes per unit dose of mutagen with least possible deleterious effects. Therefore, the usefulness of any mutagen in genetic improvement depends largely on its effectiveness and efficiency. Mutagenic effectiveness is a measure of frequency of mutations induced by unit dose of a mutagen, while mutagenic efficiency reveals an idea of the proportion of mutations in relation to associated undesirable biological effects e.g., gross chromosomal aberrations, lethality and sterility induced by the mutagen in question [3]. The most effective mutagen may not be the most efficient. In other words, different mutagens can induce same frequency of mutation per unit dose (equieffective), but they may differ in the production of undesirable changes. The present pursuit, therefore, aimed to study relative effectiveness and efficiency of γrays, EMS, NG and their combinations in grasspea as test material.

MATERIALS AND METHODS

800 seeds of grasspea, cv. Dhenkanal local were treated with each of γ-rays (5 and 10-100kR with 10kR increments), EMS (0.1-1.0 % with increment of 0.1), NG (0.005-0.050% with increment of 0.005) and combinations of 10, 40 and 70kR γ-rays with 0.2% EMS and/or 0.01% NG. γ-ray treatments were given in the ^{60} Co cell at IARI, New Delhi and chemical treatments were administered for three hours on a mechanical shaker at 28 ± 1°C after 10.5 hours of pre-seaking of seeds in distilled water. A similar lot of seeds were used as control. 500 seeds from each treatment were placed equidistantly on five petri dishes (as five replicates) and observation on germination of treated seeds was recorded after seven days of treatment. Records on root and shoot growth were noted on ten randomly selected seven days old seedlings from each

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treatment over five replications. Physiological injuries, e.g., root tip buldging and bent root tip were scored based on visual observation. The experiment was repeated twice. Rest 300 seeds of each treatment including control were sown in the field in three replications following randomized block design (RBD). M_1 plants that exhibited chimaera and seedling abnormalities with respect to leaflet number, leaflet shape and leaflet injuries etc. including albino were scored. Three matured flowers (before anthesis) were collected from first inflorescence of each of ten random plants in each treatment and around 2000 pollen grains were observed for pollen sterility. Plant survival at maturity was recorded as percentage of seeds sown in the field.

M_2 generation was raised on M_1-plant to row basis and chlorophyll and morphological mutants were scored togather as percentage of M_2 population for estimation of mutation frequency. Mutagenic effectiveness and efficiency were calculated according to Konark *et al.* [3].

RESULTS AND DISCUSSION

Induced mutagenesis has been successfully employed to generate variability and isolate valuable mutants of agronomic importance including yield *per se* [4] in many crop plants. It is in vogue to assess the genetic effects of mutagenic treatments based on chlorophyll mutation frequency in M_2 [5, 6].

Table 1: Mutagen effectiveness and efficiency in *Lathyrus sativus*

		Mutagen efficiency								
Treatment	Mutagen effectiveness[1] (M/kR, M/tc or M/kR+M/tc)	Germi-nation	Shoot length	Root length	Bent root tip frequency	Root tip buldging frequency	Pollen sterility	Chimaera frequency	Seedling abnormality	Plant survival
1	2	3	4	5	6	7	8	9	10	11
5kR	0.097	0.099	2.865	0.014	-	0.059	0.541	0.168	0.811	0.090
10kR	0.060	0.061	0.111	0.016	-	0.052	0.126	0.140	0.464	0.051
20kR	0.057	0.092	0.030	0.021	-	0.135	0.081	0.129	0.273	0.077
30kR	0.068	0.103	0.041	0.033	0.678	0.226	0.074	0.212	0.263	0.088
40kR	0.109	0.125	0.067	0.056	0.700	0.227	0.138	0.331	0.240	0.103
50kR	0.100	0.108	0.075	0.063	0.626	0.249	0.117	0.385	0.191	0.099
60kR	0.139	0.148	0.121	0.101	0.937	0.359	0.144	0.618	0.219	0.134
70kR	0.147	0.138	0.143	0.124	1.033	0.428	0.159	0.800	0.235	0.128
80kR	-	-	-	-	-	-	-	-	-	-
90kR	-	-	-	-	-	-	-	-	-	-
100kR	-	-	-	-	-	-	-	-	-	-
EMS:										
0.1%	2.210	1.326	0.780	0.019	-	0.033	0.189	0.737	2.210	0.603
0.2%	1.570	0.377	0.058	0.020	0.448	0.024	0.115	0.724	1.047	0.349
0.3%	2.024	0.209	0.052	0.031	0.372	0.039	0.158	0.520	0.651	0.158
0.4%	2.458	0.158	0.088	0.047	0.383	0.050	0.230	0.567	0.557	0.139
0.5%	3.403	0.151	0.116	0.069	0.587	0.075	0.321	1.042	0.654	0.136
0.6%	2.958	0.122	0.108	0.071	0.532	0.072	0.259	0.086	0.507	0.112
0.7%	2.654	0.114	0.094	0.074	0.546	0.071	0.220	1.161	0.497	0.098
0.8%	2.786	0.107	0.114	0.085	0.535	0.085	0.229	1.715	0.510	0.097
0.9%	2.545	0.096	0.095	0.087	0.537	0.084	0.183	2.290	0.458	0.076
1.0%	-	-	-	-	-	-	-	-	-	-
NG:										
0.005%	163.867	0.197	0.075	0.055	0.197	-	0.186	0.447	0.768	0.175
0.010%	101.700	0.144	0.081	0.053	0.049	-	0.156	0.508	0.202	0.143
0.015%	90.844	0.113	0.069	0.054	0.047	-	0.116	0.517	0.221	0.103
0.020%	108.633	0.149	0.108	0.080	0.073	-	0.134	0.716	0.322	0.132
0.025%	102.560	0.125	0.130	0.086	0.077	-	0.111	0.855	0.342	0.113
0.030%	102.800	0.123	0.133	0.103	0.092	-	0.126	1.051	0.279	0.098
0.035%	-	-	-	-	-	-	-	-	-	-
0.040%	-	-	-	-	-	-	-	-	-	-
0.045%	-	-	-	-	-	-	-	-	-	-
0.050%	-	-	-	-	-	-	-	-	-	-
Gamma ray+ EMS										
10kR+0.2%	4.003	0.552	0.496	0.219	-	0.028	0.365	0.444	2.832	0.216
40kR+0.2%	11.163	0.423	0.709	0.378	-	0.073	0.325	0.437	1.118	0.269
70kR+0.2%	19.355	0.284	0.755	0.373	-	0.117	0.291	1.097	0.840	0.215
Gamma ray+NG										
10kR+0.01%	131.794	0.475	0.475	0.234	0.202	-	0.386	0.475	0.580	0.259
40kR+0.01%	265.432	0.381	0.712	0.321	0.281	-	0.272	0.382	0.647	0.227
70kR+0.01%	415.178	0.259	0.713	0.332	0.343	-	0.275	0.841	0.724	0.215
Gamma rays+EMS +NG:										
10kR+0.2%+0.01%	207.137	4.559	3.899	1.058	-	0.060	1.559	0.478	11.854	0.956
40kR+0.2%+0.01%	353.612	1.442	3.505	1.267	-	0.101	0.765	0.429	3.257	0.507
70kR+0.2%+0.01%	493.631	0.649	2.974	1.148	-	0.141	0.465	0.819	1.355	0.422

[1]M-Mutation frequency, t-chemical mutagen treatment hours, c-concentration of chemical mutagen, kR-unit dose of gamma-ray.

But, this requires advancing M_1 to M_2 generation over a large area and a tidius effort to score all types of chlorophyll mutations many of which eventually die within seven days of seeding emergence. In this paper, the authors stressed the importance of various seedling traits even in M_1 to evaluate mutagenic treatments in inducing mutations.

Mutagenic effectiveness increased with increase in dose at higher doses of γ-rays; while in EMS and NG, it remained either same or decreased at higher concentrations (Table 1). In contrast, at lower doses, there was progressive decrease in mutagen effectiveness in all the mutagens as reported also by Gupta and Yashvir [7] in foxtail millet. However, available report [8] revealed higher mutagenic effectiveness at lower doses of γ-ray. EMS and NMU (N-nitroso-N-methyl urea) in grasspea. Combination treatments showed exceptionally high mutagen effectiveness as compared to their single treatments.

In general, mutagen efficiency based on seedling growth, bent root tip, root tip buldging, pollen sterility and chimaera frequency increased at higher doses of γ-ray. Mutagen efficiency of EMS and NG based on germination and survival concomitantly decreased and that of γ-ray increased regularly with the increase in dose. Seeding abnormality showed, more or less, decreasing trend for mutagen efficiency. These above observed differences in mutagen efficiency could be ascribable to the differences in mutagenic damage to the varied M_1 parameters. In some instances, mutagen efficiency decreased at lower doses as in the case of shoot growth and seedling abnormality in γ-ray; and pollen sterility and chimaera frequency in γ-ray and EMS. In such cases, the physiological damage to M_1 plants increased with increase in mutagen concentration at faster rate than mutation [3, 9, 10]. However, mutagen efficiency based on root growth has shown proportionate increase with dose irrespective of the mutagens used. This corroborates to the reports of Subramanian [11]; and Ignacimuthu and Babu [12]. Hence, root length may be taken as a reliable criterion for estimation of mutagen efficiency and may help greatly in planning mutation experiment even at seedling stage leading to recovery of higher mutation frequency. Tripathy and Samolo [13] stressed seedling characters, e.g., seedling height and lethality as more reliable parameters for study of mutagen efficiency than pollen sterility in mung bean. Gaul [14] observed correlation between seedling damage and mutation frequency at lower doses but not in higher doses.

Using the same set of material, Tripathy [15] reported LD_{50} of γ-ray, EMS and NG were around 50kR, 0.7% and 0.02% respectively based on germination and survival. In the present investigation, NG was found to be about 40 times effective than EMS and 1000 times effective than γ-ray at above comparable doses. NG emerged also as more efficient, while EMS and γ-ray seemed to be equi-efficient at above comparable doses. Tripathy and Samolo [13] reported more effectiveness of EMS than γ-ray and also both to have similar efficiency in mung bean. However, Waghmare and Mehra (5) reported that EMS is highly effective as well as more efficient than γ-ray in inducing chlorophyll mutations in *Lathyrus sativus* L.

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