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Mutagenic Effectiveness and Efficiency of Gamma Rays Ethyl Methane Sulphonate and Their Combined Treatments in Cowpea (*Vigna unguiculata* L. Walp)

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Abstract: Mutagenic effectiveness and efficiency of gamma rays, EMS and their combined treatments were studied in the genotype of cowpea variety CO7. The mutagenic treatments seeds were tested for lethal dose 50 percent for all mutagens, separately and the dose at which 50 percent of the seed germination was considered as LD_{50} values. Gamma rays, EMS and combined mutagens are produced a high frequency as well as a wide spectrum of mutation. The frequency of mutation was more in combined treatments than gamma rays and EMS. The mutagenic effectiveness and efficiency was calculated based on biological damage. In M_1 generation based on seed lethality (L) and seedling injury (I) and M_2 generation was carefully screened for various chlorophyll and viable mutations. Mutagenic effectiveness and efficiency increased with the decreased in dose or concentration. In the present study EMS was provide to be more effective and efficient in causing mutations as compared to gamma rays and the combined treatments.

Key words: Mutagens · Effectiveness · Efficiency · Gamma rays · EMS

INTRODUCTION

Cowpea (*Vigna unquiculata* L. Walp) is one of the world's dicotyledonous leguminous food crops and a major food crop of millions of people in the developing countries [1]. Cowpea young leaves, pods and pea contain vitamins and minerals which have fuelled its usage for human consumption and animal feeding. The roots of the cowpeas are eaten in Sudan and Ethiopia and the scorched seeds are occasionally used as a coffee substitute [2]. Cowpea grain contains about 25% protein and 64% carbohydrate [3]. The protein in cowpea seed is rich in amino acids, lysine and tryptophan, compared to cereal grains. Cowpea provides an extremely significant portion of the dietary protein of the people and plays an important nutritional role in developing countries of the tropics and subtropics, especially in sub-Saharan Africa [4].

Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like, morphology, cytogenetics, biotechnology and molecular biology etc. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement [5] and an efficient means supplementing existing germplasm for cultivar improvement in breeding program's [6]. Mutation is a sudden heritable change in organism generally the structural change in gene. It's produced by change in the base sequence of genes and it can be induced either spontaneously or artificially both in seed and Vegetative Propagated crops. Induced mutations have recently become the subject of biotechnology and molecular investigation leading to description of the structure and function of related genes. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops [7]. These mutations provide beneficial variation for practical plant breeding purpose. During the fast seven decades, more than 2252 mutant varieties have been officially released in world [8]. A great majority of mutant varieties (64%) were developed by the use of gamma rays [9]. In India still today there are 7 mutant varieties of cowpea released by both physical and chemical mutagens [10]. Hence, mutation-breeding programme has proved to be a successful tool in bringing amelioration in self-pollinated crops.

Shah *et al.* [11] reported that mutagens may cause genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants. Among the chemical mutagens, EMS is reported to be the most effective and powerful mutagen

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[12, 13]. In plants, EMS usually causes point mutations [14]. Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissue [15]. Khatri *et al.* [16] reported that gamma rays and EMS could be fruitfully applied to develop new varieties with high yield and other improved organic traits. Ethyl methane sulphonate have been found more effective and efficient than physical mutagens in crops like cowpea [17], Lentil [18]. EMS induce a high rate of mutations in both micro and higher organisms [19] and sometimes the mutation frequencies exceed those obtained by radiation [20]. The usefulness of a mutagens in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagens), but also on its mutagenic efficiency (mutation in relation to undesirable changes like sterility, lethality, injury etc.). The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations [21] and [22]. The two mutagens acting in a sequence one after the other may produce more than additive effect, if the sites not affected by the first are exposed to the action of the second. On the other hand, if two mutagens merely compete for the same site, or if their actions are independent, the results obtained will be additive or less than additive [23], such studies besides, enhancing or reducing mutation frequency, would also help in fixing most effective combination treatments. The present investigation was undertaken to study the frequency and spectrum of macro mutations along with the mutagenic effectiveness and efficiency of different doses of gamma rays, EMS and combined treatments were undertaken in cowpea.

MATERIALS AND METHODS

The dry and dormant seeds of the cowpea (*Vigna unguiculata* L.walp) variety CO-7 were treated with gamma rays, EMS and their combination treatments were used in the present study. 300 well filled healthy seeds packed in moist germination paper were selected for each treatment in the gamma chamber at 15, 20, 25, 30 and 35 KR doses of gamma rays in ^{60}CO gamma source (irradiation source capacity to release 3000 Ci delivery 7200 r/min). The gamma irradiation was carried out at sugarcane breeding institute (ICAR), Coimbatore, India. Similarly, in case of EMS treatment individually and in combination with gamma rays. 300 healthy seeds each were presoaked in distilled water for 6 hours at room temperature. For EMS treatment, the presoaked seeds were treated with 5, 10, 15, 20 and 25mM freshly prepared solution for 3 hours. For combination treatments 300 seeds each were first irradiated with gamma rays at 15, 20, 25 30 and 35 KR doses and then followed by EMS, only one concentration of EMS (15mM) was used in combination with 15, 20, 25 30 and 35 KR gamma rays. After the EMS treatment, the treated seeds were washed thoroughly for 1h in running tap water to terminate the residual effect of the mutagenic chemicals.

After the completion of the treatment the treated seeds were sown immediately in the field along with their respective controls to raise the M_1 generation in a randomized block design with three replications. All the treatments including the controls were raised adopting a spacing of 45 cm in between rows and 20cm in between plants. All the recommended cultural measures namely, irrigation, weeding and plant production methods were carried out during the growth period of the crop.

The seedling height reduction (I) in different M_1 generation was studied following Nilan *et a.* [24] Sharma [25] and velu *et al.* [26]. The plant survival (L) was computed as the percentage of plants surviving till maturity. The biological damage (lethality/ injury) was computed as the reduction in plant survival and plant height. At maturity all the surviving M_1 fertile plants were harvested separately and seeds were sown in the next season in plant progeny rows to raise M_2 generation. The respective control and treatment progenies were screened several times for morphological mutations throughout the crop duration. Different kinds of chlorophyll mutants (Xantha, viridis, chlorina and albina) were scored from emergence till the age of four week in M_2 generation by using modified classification of Lampretcht [27] and Kharkwal [28]. Mutation frequency was calculated as percentage of mutated M_2 progenies for both chlorophyll and morphological mutations in each treatment. The Mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak *et al.* [29].

Mutagenic effectiveness (Physical mutagens) = Mf×100/krad.

Mutagenic effectiveness (Chemical mutagens) = Mf×100/c×t.

Mutagenic effectiveness (Combined mutagens) = Mf×100/c×t× krad.

M = Mutation frequency for 100 M_2 plants
t = Period of treatment with chemical mutagen in hours
C = Concentration of mutagen in mM in percent
Krad = dose of mutagenic radiation in kilo rad
L = Percentage of lethality (or) survival reduction
I = Percentage of injury (or) reduction in seedling size.

RESULT AND DISCUSSION

LD_{50} value was calculated on the basis of 50 percent reduction of germination seeds count on 10^{th} day. The present investigation exhibited that the germination percentage of cowpea decreased with the increase in the Dose or concentration of the mutagens were used to find out the LD_{50} values for further studies. It was estimated that using 50% reduction in seed germination observed at 25KR gamma rays, 15mM of EMS and 25KR+15mM of combined treatments. The LD_{50} value for EMS was observed between 15 and 20mM concentration for both germination and survival and it coincides with the report of Thirugnanakumar [30] in cowpea. The LD_{50} value for gamma rays was observed between 20 and 25KR of for cowpea, mungbean and Bengal gram as reported by Palaniswamy [31], Louis kadambavana sundaram [32] and Vadivelu [33] respectively.

The impact and the tolerance level of the biological material to a mutagen are manifested in M_1 generation itself in terms of germination and lethality [34]. In the present investigation germination and survival percentage decreased with increasing dose/ concentration and a field condition was observed in M_1 generation. Similar results were observed by Dhanavel *et al.* [35] and Kavithamani *et al.* [36].

The frequency of chlorophyll and viable mutants observed in M_2 generation is mainly used as a dependable measure of genetic effect in mutagen [37, 38]. The mutation frequency showed a decrease with increase in the dose or concentration of mutagens. In the present investigation, the maximum chlorophyll and viable mutation frequency observed at 15mM of EMS (3.26). While the minimum chlorophyll and viable mutation frequency was observed at 30KR of gamma rays (1.03) (Table-2).

Chlorophyll Mutant: The spectrum of chlorophyll mutant's viz., albina, virescence and xantha were observed at all mutagenic treatments.

Albina: These mutant leaves were white in color, due to absence of all pigment. This was leaded to the death of the plants at 10-15 days after germination.

Virescence: These mutants showed leaf margin more segregated as compared to control. Young leaves were pale green in color during maturity time. One or two mutants were observed at all mutagenic treatments.

Xantha: The leaves turned yellow in color due to the absence of xanthophylls.

Keeping in view, it was observed in M_2 generation that EMS was more pronounced in inducing chlorophyll mutations than gamma rays [39] and among the spectrum, the viridis (less drastic mutation) was more than that of albina (extreme mutation) as categorized by westergaard [40]. Similar observations were made by Packiaraj [41] and Rangaswamy [42] in cowpea; Mehraj-ud-din *et al.* [43]; Deepalakshmi and Anandakumar [44]; Singh and Mohapatra [45] in black gram; Solanki [46] in lentil.

Table 1: Determination of Ld_{50} Value for Gammarays, Ems and Combined Treatments

Treatment Dose/Conc.		Seed germination (%)	Percent of reduction over control
Control		97.00	100.00
Gamma rays (KR)	15	71.00	27.79
	20	62.33	36.62
	25	49.66	49.50
	30	35.66	63.74
	35	24.33	75.26
EMS(Conc. mM)	5	76.33	21.85
	10	61.66	36.87
	15	52.33	46.42
	20	41.00	58.32
	25	32.33	66.90
Combined treatment (Gammarays +EMS)			
	15+15	74.66	23.03
	20+15	64.33	33.69
	25+15	53.66	44.69
	30+15	41.66	57.06
	35+15	33.66	65.30

Table 2: Frequency of Chlorophyll and Viable Mutants in M_2 Generation

Treatment Dose/Conc.		Total plants studied in M_2 generation	Total plant segregated in M_2 generation	Mutation frequency
Gamma rays (KR)	15	520	4	0.74
	20	780	9	1.15
	25	1000	17	1.70
	30	675	7	1.03
	35	430	4	0.93
EMS(Conc. mM)	5	575	5	0.86
	10	730	13	1.78
	15	950	31	3.26
	20	645	12	1.86
	25	470	8	1.70
Combined treatment (Gammarays +EMS)				
	15+15	510	5	0.98
	20+15	720	15	2.08
	25+15	788	25	3.17
	30+15	630	9	1.42
	35+15	500	7	1.40

Table 3: Mutagenic Effectiveness and Efficiency in M_2 Generation

Treatment Dose/Conc.		Survival Reduction (Lethality) (%)	Height Reduction (Injury) (%)	Mutation Frequency	Effectiveness $\frac{M \times 100}{KR(or)C \times T}$	Efficiency $\frac{M \times 100}{L}$	Efficiency $\frac{M \times 100}{I}$
Gamma Rays (KR)	15	33.68	20.72	0.74	5.06	2.19	3.57
	20	36.61	30.00	1.15	5.75	3.14	3.83
	25	42.34	39.55	1.70	6.80	4.01	4.29
	30	50.08	50.55	1.03	3.43	2.05	2.03
	35	51.90	55.68	0.93	2.65	1.73	1.67
EMS (Conc. mM)	5	35.94	16.31	0.86	5.73	2.39	5.25
	10	39.40	24.51	1.78	5.93	4.51	7.26
	15	44.01	30.96	3.26	7.24	7.43	10.52
	20	50.94	35.88	1.86	3.10	3.65	5.18
	25	53.64	39.87	1.70	2.26	3.16	4.26
Combined Treatment	15+15	38.96	19.31	0.98	0.78	2.51	5.07
(Gamma rays + EMS)	20+15	41.34	26.38	2.08	0.83	5.03	7.88
	25+15	47.45	30.96	3.17	0.84	6.68	10.23
	30+15	54.88	37.91	1.42	0.28	2.58	3.74
	35+15	56.52	43.80	1.40	0.22	2.47	3.19

Viable Mutant: Gaul [47] classified viable mutations as macro and micro mutations, while Swaminathan [48] grouped them as macro mutations and systematic mutations. The mutational event may be e accompanied by a large or small change in phenotype. Such changes have the highest significance in plant breeding and have been stressed by several authors [49, 50]. In the present investigation, some of the morphological (viable) mutants were observed in M_2 generation with different dose or concentration of gamma rays, EMS and combined treatment an increase in the number of viable mutants were realized in the present study. 15mM of EMS produced more number of viable mutants than gamma rays and EMS.

Tall and dwarf mutants were observed in different mutagenic treatments. Among the dose or concentration of maximum number of mutants were recorded at 25 KR of gamma rays. Similar mutants were observed by Sinha [51] and Juliet Hepziba and Subramanian [52] in black gram, Kumar and Dubey [53] in *Lathyrus sativus,* Ramesh and Seetharami Reddi [54] in rice, Yadava *et al.* [55] in kodo-millet and Pavadai 2006 in soybean.

The leaf mutant such as tetra foliate leaf and penta foliate leaf were observed in different mutagenic treatments. Among the dose or concentration maximum number of leaf mutants was recorded at 20KR+15mM combined treatments. Similar mutants were observed by Sengupta and Datta [57] in sesame.

The pod mutants such as single seeded pod and long pod were observed in different mutagenic treatments. The high number of pod mutant was observed at 25 KR+15mM of combined treatments. Similar results were observed in Kumar and Dubey [53] in *Lathyrus sativus* and Prasad and Das [58], Vandana *et al.* [59], Vandana and Dubey [60] in lentil and Juliet Hepziba and Subramanian [52] in black gram, Pavadai [56] in soybean.

The brown and white colour seed mutants and bold size seed mutants were observed at all mutagenic treatments. Similar reports of seed colour were isolated by Prabhakar [61], Pugalendi [62] and Ganesan [63] in sesame, Verma and Raj [64] in mustard and Pavadai [56] in soybean.

Physiological mutants such as early and late maturity were observed in all the mutagenic treatments. The maximum number of early and late maturity mutant was observed at 15mM of EMS. Early maturity mutant were reported by Kumar and Dubey [53] in *Lathyrus sativus,* Reddy [65] and Pugalendi [65] in sesame, Yadava *et al.* [55] in Kodo-millet, Sasi [66] in bhendi and Pavadai [56] in soybean.

Mutagenic Effectiveness and Efficiency: Mutagens induce differential genetic and cytogenetic changes [67]. Thus the mutagenic effectiveness and efficiency will also depend upon the nature of induced mutations. In case of sparsely ionizing radiations like gamma rays, the ratio of point mutations to chromosomal aberration in much higher than observed in densely ionizing radiations. Sterility induced by chemical mutagens, more particularly alkylating agents in many cases was not found to be associated with chromosomal abnormalities [68]. Prasad [69] observed that NMU induced minimum visible mitotic changes in *Tritium durum*, but resulted in a very high degree of sterility. It appears that the gene mutations may be responsible for such sterility although cytological undetectable cryptic structural changes may also contribute to some extent.

In order to obtained high effectiveness and efficiency, the mutation effect must greatly surpass other effects in the cell such as chromosomal aberrations, physiological and toxic effects, which reduce cell survival and eliminate the mutation. Both mutagenic effectiveness and efficiency generally decreased with the increasing dose or concentration.

The mutagenic effectiveness and efficiency of mutagens was estimated on the basis of relative propagation of families segregating chlorophyll and viable mutations. In comparing effects of physical, chemical and combined mutagens reduction in plant survival and plant height were observed (Table 3).

Mutagenic Effectiveness: Effectiveness means the rate of mutation induction as dependent upon the mutagenic does and efficiency refers to the mutation rate in mutation to the various biological effects usually a measure of damage [24]. In general the effectiveness decreased with increasing dose or concentration. With increasing doses of EMS or Gamma rays the values obtained for all the biological criteria for M_1 generation were decreased. The reduction in biological criteria (Plant height and Survival) may be attributed to a drop in the auxin level [70], inhibition of auxin synthesis [71], Chromosomal aberrations [72] or due to decline of assimilation mechanism [73]. EMS was found to be more effective than gamma rays and combined treatments in inducing mutation. The maximum mutagenic effectiveness was observed at 15 mM of EMS (7.24) and the minimum mutagenic effectiveness was observed at 35KR+15 mM of combined treatments (0.22). Similar results were recorded by Gautam *et al*. [74] and Solanki and Sharma [75] in mung bean; Solanki [46] in lentil; Yadava *et al*. [55] in kodo miilet; Kharkwal [28] and Jabee and Ansari [76] in chickpea.

Mutagenic Efficiency: The mutagenic efficiency was worked out based on injury and lethality. Efficiency of a mutagenic agent is of a complex nature, as it dose not depends on the reactivity of the agent with the material and on its applicability to the biological system but also on the degree to which physiological damage, chromosomal aberration and sterility are induced in addition to mutations. The mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagen [29]. Efficient mutagens and their treatments are indispensable for the cost-effective use of the mutagen as a tool for the induction of mutations and their direct and indirect utilization in successful breeding programme.

On the basis of lethality, the highest mutagenic efficiency was recorded at 15mM of EMS (7.43) and the lowest mutagenic efficiency was observed at 35KR of gamma rays (1.73). On the basis of injury, the maximum mutagenic efficiency was observed at 15mM of EMS

(10.52). The minimum mutagenic efficiency was observed at 35 KR of gamma rays (1.67). In general the mutagenic treatment 15mM EMS was found to be highly efficient to induce chlorophyll and viable mutants. Similar results recorded by Gautam *et al.,* [74] in mungbean; Ahmed [77], Deepalakshmi and Anandakumar [44] and Sharma *et al.,* [78] in urdbean; Jayakumar and Selvaraj [79] in sunflower, Yadava *et al.,* [55] in kodo-millet and Jabee and Ansari [76] in chickpea.

In conclusion, the improved variety of CO7 responded more and more number of viable and economic mutants for higher productivity. EMS was proved to be more effective and efficient than gamma rays and combined mutagens.

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