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Physical and Chemical Mutagenesis for Improvement of Chilli (*Capsicum annuum* L.)

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Abstract: A comparison of the effectiveness and efficiency of gamma rays and ethyl methanesulfonate (EMS) for inducing mutation in chilli seeds of 10 different doses along with one respective control. Seeds were treated with 10, 20, 30, 40 and 50 kR of gamma rays and 10, 20, 30, 40 and 50 mM of ethyl methanesulfonate. The M_1 generation was produced from these mutagen treated seeds. Several unique and interesting chlorophyll and viable mutants were obtained in the M_2 generation (subsequent generation derived from the seeds of M_1 generation). Chlorophyll mutations are used to evaluate the genetic effects of various mutagens. The spectra of chlorophyll mutants albina, chlorina, viridis and lutescens were most frequently. In M_2 generation, gamma rays induced higher proportion of chlorophyll mutants than EMS. Frequency of viable mutants was, in general, higher in treatments with EMS than with gamma rays. Treatment with 30 mM EMS was more effective in inducing desirable mutations at the highest frequency. The desirable viable mutants of long and dark red pods, base non-bulging fruits, flower mutant (normally pentamerous flower but an abnormal behavior of trimerous, tetramerous, heptamerous), two or three flowers on a peduncle, profuse branching and pod setting and genic male sterility were isolated from different treatments. Lethality or biological injury, apparent as reduced germination, increased with increasing dose of gamma rays and EMS. Mutagenic effectiveness and efficiency generally increased with increasing dose of EMS. Mutation breeding will significantly increase the chilli amelioration both the effectiveness and efficacy of induced variability of desirable traits to develop improved genotypes.

Key words: *Capsicum annuum* · Induced mutation · Ethyl methanesulfonate · Pentamerous · Efficiency · Effectiveness

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the most cultivated vegetable spice crops in tropical and subtropical climates. India is the largest consumer and exporter of chillies in the International markets and exports dry chilli, chilli powder and oleoresins to over 90 countries [1]. The production of chilli in India is dominated by Andhra Pradesh which bestows 53% to the total area production. It is grown in several parts of India has a larger area, its productivity is low when compared to other countries. Hence, there is an urgent need to produced and identify new varieties combining high level of disease resistance, besides increased yield, capsaicin content in chilli.

Mutations are the tools and being used to study the nature and basis of plant growth and development, thereby producing raw materials for genetic improvement of crops [2]. Induced mutations can rapidly create variability in quantitatively and qualitatively inherited traits in crops [3 and 4]. Mutagenesis is one of the most critical steps for genetic studies as well as selective breeding. Various m utagenic agents are used to induce favourable mutations at high frequency that include ionizing radiation and chemical mutagens [5]. Successful mutant isolation largely relies on the use of efficient mutagens. In plant research, a chemical mutagen, ethyl methanesulfonate (EMS) produces single base substitutions with different mutation spectra. These chemo mutagens induce a broad variation of

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morphological and yield structure parameters in comparison to normal plants [6]. The present study was undertaken to gather information on the response of chilli to doses of irradiation and chemical mutagens and determine the type and frequency of mutations.

MATERIALS AND METHODS

Chilli var.K1 was irradiated with 10, 20, 30, 40 and 50 mM with a ^{60}Co gamma cell, at Sugarcane Breeding Institute, TNAU, Coimbatore, India. Another quantity of 5gram seeds for each treatment was presoaked for 12h in distilled water, blotted dry and treated with 10, 20, 30, 40 and 50 mM of freshly prepared solutions of ethyl methanesulfonate for 4h with intermittent shaking. After treatment, seeds were thoroughly washed in running water for 4h to leach out the residual of chemicals. The treated seeds were sown in seed beds and watered at least once a day. After 25-30 days, seedlings were shifted to new pots as one plant per pot. The M_1 generation (produced directly from mutagen treated seeds) was grown in the pot culture experiment at the Botanical Garden, Department of Botany, Annamalai University. All the recommended cultural practices were carried out during the plant growth period.

All surviving M_1 plants were selfed and harvested to form M_2 generations. From each treatment in M_1, a total of 275 seeds in ten sets with one control (untreated) were placed on moistened germination paper to determine lethality on the basis of seed germination were noted from emergence until 2-3 weeks after germination. Chlorophyll mutations, as described by [7] were scored throughout the plant growing period. Mutations affecting gross morphological changes in growth habit, number of leaves, number of flowers and flower development, pod shape, size and color classified as viable macro mutations. Mutagenic effectiveness and efficiency were calculated as per [8] where:

Mutagenic effectiveness (Gamma rays) = Mutation rate (M_2 family basis)/Dose in kilo Roentgen (kR)

Mutagenic effectiveness (EMS) = Mutation rate/conc. of EMS in mM × time in hours and Mutation efficiency = Mutation rate/percentage lethality or biological injury in M_1

RESULTS AND DISCUSSION

Mutation frequency is the observed or estimated number of mutations at a given mutagen dose per population of cells, gametes, plants or plant parts at a specific mutant generation [9]. Mutation frequency can be calculated as a percentage of mutants from the irradiated seeds (M_0), surviving seedlings in the first mutant generation (M_1) or mutated plants in the second mutant generation (M_2) analysed [10]. Mutation frequency as the frequency at which a specific kind of mutation (or mutant) is found in the population of cells or individuals [11]. This definition disregards the number of mutations that give rise to the desired mutant. However, within in one cell, more than one independent mutational event can take place. But on the other hand, not all genes are expressed in each cell at each moment. The frequency and saturation of mutations can be regulated by varying the mutagen dose [12, 13] and mutagenic agents can induce different extensions of genomic lesions, ranging from base mutations to larger fragment insertions or deletions [14, 15]. The frequency of chlorophyll and viable mutants observed in M_2 generation is mainly used as a dependable measure of genetic effect in mutagen [16 and 17]. The isolation of chlorophyll mutations was, in general significantly higher in M_2 generations as gamma irradiation dose than EMS rate increased (Table 1 and 2). The results are similar to those of [18] in blackgram and [19] in garden pea. The frequency of viable mutants was, in general, higher in treatments with EMS than with gamma rays.

Chlorophyll Mutations: Mutation frequency to be estimated on the basis of phenotype, using screens for seedling lethality (survival rate), embryonic lethality (seed set), chlorophyll deficiency or single-copy gene phenotypes as a measure. Frequencies and types of the chlorophyll mutations albino, chlorina, viridis and lutescens varied. Generally, gamma rays induced higher proportion of chlorophyll mutants than EMS. In *S. villosum*, the earliest observable mutants were chlorophyll deficiency mutants [7] whose frequencies varied with the mutagen dose and male sterile mutant frequency was later observed to follow a similar distribution trend [20]. Although, the chlorophyll mutations do not have any economic value due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability.

Table 1: Mutation rate of various doses of gamma rays and EMS in M_2 generation

Mutagen			Chlorophyll mutants							
			M_2 families basis		M_2 plants basis		Viable mutants		Total mutants M_2 plant basis	
Gamma rays (kR)	Number M_2 families	Number M_2 plants	No.	%	No.	%	No.	%	No.	%
10	198	845	14	7.07	27	3.19	5	0.59	32	3.78
20	212	916	18	8.49	25	2.73	6	0.66	31	3.38
30	196	813	21	10.71	29	3.57	10	1.23	39	4.80
40	143	758	7	4.89	17	2.24	7	0.92	23	3.03
50	121	445	3	2.48	10	2.25	2	0.45	12	2.70
EMS(mM)										
10	167	917	14	8.38	19	2.07	5	0.55	24	2.62
20	190	943	11	5.79	19	2.01	9	0.95	28	2.97
30	207	828	12	5.80	13	1.57	14	1.69	27	3.26
40	167	844	5	2.99	8	0.94	7	0.83	15	1.78
50	153	510	2	1.31	5	0.98	4	0.78	9	1.76

Table 2: Spectrum and frequency of induced chlorophyll mutations in M_2 generation

Mutagenic treatments	Albino (%)				Chlorina (%)				Virescent (%)			
	M_2 families		M_2 plants		M_2 families		M_2 plants		M_2 families		M_2 plants	
dose/conc.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Gamma rays (kR)												
10	2	1.01	5	0.59	3	1.52	7	0.82	4	2.02	13	1.54
20	2	0.94	6	0.66	2	0.94	5	0.55	4	1.89	7	0.76
30	3	1.53	8	0.98	4	2.04	15	1.85	3	1.53	11	1.35
40	4	2.79	4	0.53	4	2.79	19	2.50	4	2.79	11	1.45
50	3	2.48	7	1.57	3	2.48	20	4.49	4	3.31	13	2.92
EMS(mM)												
10	-	-	-	-	3	1.80	7	0.76	1	0.59	3	0.32
20	1	0.52	6	0.64	3	1.58	10	1.06	4	2.11	10	1.06
30	3	1.44	6	0.72	4	1.93	11	1.33	5	2.42	9	1.09
40	3	1.79	11	1.30	3	1.80	18	2.13	3	1.80	11	1.30
50	2	1.31	8	1.57	3	1.96	10	1.96	4	2.61	12	2.35

Frequency and spectrum of chlorophyll mutations increased as irradiation and chemical mutagen doses increased (Table 2). Some important types of chlorophyll mutants are described below.

Albino: neither carotenoids nor chlorophyll is formed.

Viridis: young leaves are yellowish, but later change to normal green [21 and 22]. These can further be subdivided into:

Virescens: light green gradually changing to dark green and mostly viable.

Chlorina: greenish yellow variegation or chlorophyll deficiency [23].

Lutescens: cotyledons and leaves are uniformly yellowish, lighter than normal green; however, distinct accessions may represent different intensities of color [24-26].

Mutation spectra can be altered in various ways by the application of different mutagens. This is valid not only with regard to the types of chlorophyll mutations but also with regard to the rates of translocation sterility and viable mutations.

Viable Mutations: The central matter in this mutation analysis concern the viable mutations, may of which, whether they are morphological or physiological in character, drastic or modifying, have potential value in plant breeding. The highest rates obtained with various mutagens as well as the corresponding kinds of treatment,

Table 3: Induced viable mutations for various traits in M_2 generation

	Gamma rays (kR)					EMS (mM)					Total viable
Trait	10	20	30	40	50	10	20	30	40	50	mutants
Tall mutant	-	1	3	1	-	2	-	5	1	-	13
Profuse branching	-	2	4	3	-	-	1	7	2	-	19
Curved leaf	1	-	4	-	-	1	-	3	-	-	9
Diminished morphology	1	-	2	2	4	-	-	1	-	-	10
Dwarf plant	1	-	4	2	2	1		1-	2	4	17
Flower mutant	-	2	6	1	-	-	2	12	4	-	27
Rosette leaf	-	-	-	-	-	-	-	2	-	-	2
Pointed fruit apex	-	1	1	1	-	-	1	5	-	-	9
Male sterile	1	-	7	4	4	-	1	6	1	3	27
Long pods	-	1	4	1	-	-	1	16	1	-	24
Dark green pods	-	-	6	2	2	-	2	14	1	-	27
Yellowish green pods	-	1	4	-	-	-	-	1	-	-	6
Total	4	8	47	17	12	4	9	67	8	7	190

are visualized. Frequency of mutants with desirable viable morphological plant and pod types occurred. The 30 mM EMS was significantly more effective in inducing desirable plant and pod type mutations when compared to gamma rays (Table 3). Some desirable types of mutants described as:

Tall Mutant: plant height, 65 to 80cm; few branches, flowers and fruits and frequency was higher with chemical mutagens than gamma irradiation.

Curved Leaf: leaves have curved leaf blades with long petioles, fewer branches, flowers and fruits; seed set poor [27].

Diminished Morphology: extremely small (2cm in length, 1cm in width) leaves with normal shape; main stem before the first cyme has 18 to 20 internodes; equally tiny stem and flowers [24 and 25].

Dwarf Plant: 15 to 20cm in height; short internodes; thick, dark green leaves [28].

Flower Mutant: generally *Capsicum* species has pentamerous flower but an abnormal behavior of trimerous, tetramerous, heptamerous nature. This is also reported mutants with change in number of sepals, petals and ovules than normal in *Borago officinalis* [29].

Rosette Leaf: the rosette plants were shorter, had smaller leaves which is dependent upon the length of the petiole and delayed floral development.

Pointed Fruit Apex: incomplete dominant to blunt [23 and 30].

Genic Male Sterility: Male sterility is utilized for economic production of hybrids on a large scale in various crops. In chilli, 30, 40 and 50 kR of gamma rays and 30 mM of EMS mainly induced genic male sterility (androecium transformed into petaloid structures). This is already reported this trait [31]. In some cases 5 anthers or 2-3 anthers are modified into petaloid structures. The colour intensity of the stamen also varied in treated plants (dark blue into yellow colour).

Fruit Base Non-bulging: Similar mutant were earlier reported by Miller and Fineman, 1938.

Long Pods: Commonly, consumers prefer long pods. A total of 16 such mutants were isolated from 30mM of EMS. These mutants had a pod length of 8 to 10.5cm (Fig. H).

Mutagenic Effectiveness and Efficiency: Mutagenic effectiveness reflects rate of mutation in relation to mutagen dose, whereas mutagenic efficiency is the mutation rate in relation to lethality or biological injury. There were differences in mutagenic effectiveness and efficiency (M/L) in relation to EMS and irradiation dose (Table 4). Lethality or biological injury based on germination, increased with increasing doses of irradiation and EMS. Mutagenesis 50 mM EMS produced the highest lethality followed by 50 kR of gamma rays. Mutagenic effectiveness decreased with increase in mutagenic dose. Similar trend of decreasing effectiveness has been reported in chickpea [32].

Table 4: Mutagenic effectiveness and efficiency of mutagens in M_2 generation

		Mutagenic effectiveness		Mutagenic efficiency	
Mutagenic Dose	Lethality (%)	M_2 families	M_2 plants	M_2 families	M_2 plants
Gamma rays (kR)					
10	42	0.71	0.32	0.17	0.076
20	45	0.42	0.14	0.19	0.061
30	52	0.36	0.12	0.21	0.068
40	33	0.12	0.06	0.15	0.068
50	20	0.50	0.05	0.124	0.112
EMS (mM)					
10	38	0.21	0.052	0.22	0.054
20	42	0.07	0.025	0.14	0.047
30	49	0.05	0.013	0.12	0.032
40	32	0.02	0.059	0.09	0.029
50	24	0.006	0.005	0.05	0.040

Mutagenic efficiency of gamma rays increase with increased dose which might be due to fewer M_2 families harvested in M_1 generation (individual plant harvested in M_1 generation forming one M_2 family and plants developed from this family are called as M_2 plants). There were differences in gamma rays mutagenic efficiency decreased with increasing EMS and gamma rays concentration. The higher mutagenic effectiveness and efficiency do not reflect per se mutation frequency and cannot be used as indices for maximization of mutation rates [32]. In general, the chlorophyll mutation frequency increased with increasing dosage upto certain limit, beyond which it exhibited a decline. This shows that a saturation point was reached at higher dose level to the rigor diplontic and haplontic selections in the irradiated materials. Efficient mutagenesis results in production of the maximum number of desirable changes accompanied by the least possible amount of undesirable change [18]. Mutagenic effectiveness and efficiency increased with increasing EMS dose with few exceptions. Mutation breeding approach can be used to induce a number of desirable traits in chilli which has been isolation of novel mutants, making a great contribution to plant genetics and breeding.

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