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Frequency in germination studies of chlorophyll mutants in effectiveness and efficiency using chemical mutagens

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ABSTRACT

The germination study was made to understand the genetic variability in *Zea mays* (L.) variety JKMH-1001which was subjected to chemical mutagenic treatments for two generations. The chemical mutagens namely, EMS, DES and Sodium azide were used. The mutagen treated seeds were tested for lethal dose of 50 per cent for all mutagens, separately and the dose at which 50 per cent of the seed germination was considered as LD_{50} values were grown in the field. The spectrum of chlorophyll mutation consisted of albino, chlorina, viridis and xandha. The morphological mutations observed at tall, dwarf, early maturity, late maturity, triangular leaf, bold size seed, long ear, short ear and male sterility in all the concentration recorded. In EMS was found to be more effective and efficient than the other mutagens. The effectiveness was producing germination and chlorophyll mutants. Effectiveness means frequency of mutations induced by unit dose/concentration of a mutagen. The efficiency means undesirable biological effect like lethality and sterility caused by the mutagen. The effectiveness and efficiency generally decreased with increased in the higher doses of the mutagens in certain level.

Introduction

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 (Acharya *et al.*, 2007) and as an efficient means of supplementing existing germplasm for the of cultivar improvement in breeding program's (Dubinin, 1961)

Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee *et al.*, 2002). 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 the world (Maluszynski *et al.*, 2000).The chlorophyll mutants in M_2 seedlings were screened from 15^{th} to 20^{th} day to record. The various chlorophyll mutants were periodically the classification and identification of the chlorophyll mutants was done based on the nomenclature adopted by Gustafsson (1940).

Corn belongs to the grass family and is a cross-pollinated, monoecious plant in which the male and female flowers are located in different inflorescences on the same stalk. Maize is a tall, annual grass with overlapping sheaths and broad conspicuously distichous blades.Maize is chiefly used as food for man and livestock. The grain is very nutritious, with a high percentage of carbohydrates, fats and proteins. Not only has the grain plant as a whole formed an important fodder crop. The immature cobs are largely eaten after roasting. The grains are also used in making corn starch and industrial alcohol. Glucose is also manufactured from the grain. The corn oil is prepared which is used for soap making, in lubrication and as salad oil. Corn flakes make a good breakfast food.

Materials and Methods

Two sets containing 200 well filled healthy seeds were selected for treatment. To determine the LD_{50} values, seeds were pre soaked in distilled water for 6 hours followed by EMS, DES and Sodium azide at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100mM concentrations. EMS, DES and Sodium azide were thoroughly washed in running tap water for 8 to 10 times and then transferred to Petri dishes containing two layers of moist filter paper for germination. Ten Petri dishes of 10 seeds per treatment were planted and percentage germination and seedling variations for each treatment were subsequently determined. The treated seeds were then subjected to germination test. Based on the reduction of germination to 50 per cent, LD_{50} value was determined. Three concentrations of EMS, DES and Sodium azide around LD_{50} were fixed for further studies.

200 well filled seeds were selected and pre soaked in double distilled water for six hours. The excess moisture in the seeds was removed by pressing in the fold of filter paper. They were treated with required mM concentrations of EMS, DES and Sodium azide solution for six hours at room temperature (26±2°C) with intermittent shaking. After that the seeds were thoroughly washed with running tap water for 8-10 times. Non-treated dry seeds were pre-soaked in distilled water for 6 hours and then used as control. The mutagenic treatment seeds were immediately sown in the field along with the control in a randomized block design with three replications. A total of 200 seeds were sown in each treatment. All the treatments including the control were raised adopting a spacing of 45 cm in between rows and 20 cm 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. From the remaining treated seeds, 100 seeds were

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placed in the moist germination paper and replicated twice for the purpose of laboratory analysis.

The seeds harvested from M_1 generation were taken from individual treatments and were used to rise M_2 generation (Panda and Subuthi, 1994). The M_2 generation was grown in triplicate in randomized block design. 200 plants were maintained for each treatment in each replication. Biometric observations were recorded and individual plant data's were calculated using statistical analysis.

In the Mutagenic effectiveness means the frequency of mutations induced by unit dose/concentration of a mutagen. The efficiency means undesirable biological effect like lethality and sterility caused by the mutagen. The effectiveness and efficiency of the mutagens namely, EMS, DES and sodium azide were worked out by using the formulae suggested by (Konzak *et al*., 1965).

Mutagenic effectiveness = $M \times 100 / C \times T$

Mutagenic efficiency (Lethal) = $M \times 100 / L$

Mutagenic efficiency (Injury) = $M \times 100 / I$

Where

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

L - Percentage of lethality or survival reduction

I – Percentage of injury or reduction in seedling size

Results and Discussion

Maize genotype was subjected to study the effect of chemical mutagens namely, EMS, DES and sodium azide through the biological changes in M_1 generation. In present study the germination, spectrum of chlorophyll in effectiveness and efficiency and total mutations in M_2 generation. It was also aimed to find out the economic potentialities of the viable mutants and the nature of induced variability in the quantitative traits in M_2 generations.

Seed germination

The germination seeds were counted from 3^{rd} to 7^{th} day recorded. The number of seeds germinated recorded in 15^{th} day after sowing in each treatment was counted and percentage was worked out due to all mutagenic treatments. The reduction in seed germination ranged from 45.70 to 67.13, percent in EMS. 41.42 To 67.71, percent in DES and 45.70 to 7142, percent in sodium azide. The LD_{50} values were calculated on the basis of 50 per cent reduction of germination seeds count on 10^{th} day. The germination percentage of *Zea mays* decreased with increase in the concentration of the mutagens was used to find out the LD_{50} values for further studies. It was estimated that using 50% reduction in seed germination observed at 50mM of EMS, 40mM of DES and Sodium azide. (Table-1). The seed germination and seedling survival were reduced with increasing in concentration of EMS, DES and sodium azide treatment than control. Similar results have been reported in different crops, Soybean (Pavadai and Dhanavel, 2004), cluster bean (Velu *et al.,* 2007), cowpea (Girija and Dhanavel, 2009).

The M_1 generation was assessed at the field level to measure the intensity of injury caused by mutagenic treatments (Gaul, 1970). The biological effects were determined from the observation made on seed germination. The seedling survival decreased in all the mutagens. In germination and survival percentage decreased with increase in concentration and a field condition was observed in M_1 and M_2 generation. Similar results were observed by soybean (Kavithamani *et al.,* 2008).

Chlorophyll mutants

Frequency of chlorophyll mutations

The chlorophyll mutations were observed in the M_2 generation were scored at the seedling in the field and expressed on M_1 panicle family basis as well as on M_2 seedling basis. In the genotype no chlorophyll mutations were observed in the control population. The treated genotype behaved differently in the frequency of occurrence of chlorophyll mutations accumulating. The chlorophyll mutants produced a higher proportion of chlorophyll mutations in EMS mutagen was more effective chlorophyll mutations.

The observed frequency of viable mutants estimated on M_2 plant basis is On M_2 plant basis, the maximum chlorophyll and viable mutation frequency were observed at 50mM of EMS (18.57). While the minimum chlorophyll and viable mutation frequencies were recorded at 30mM of Sodium azide (7.14). The frequency of chlorophyll mutants in M_2 generation is mainly used as a dependable measure of genetic effects in mutagens (Nilan and Konzak, 1961). The mutation frequency showed a decrease with increase in the concentration of mutagens. In the present investigation, the spectrum of chlorophyll mutant's *viz.,* albino, chlorina, viridis and xantha were observed (Table-2) in all mutagenic treatments.

Spectrum of chlorophyll mutations

The spectrum of chlorophyll mutations obtained in the present study induced different types, viz., albino, chlorine, xandha and viridis was observed. These types mutations observed in maize M1 and M2 generations.

Albino

These seedlings were characterized by their dull white color and were devoid of chlorophyll, carotenoide and other pigments. Albino seedlings are smaller in height and survive to a maximum of 20 days after germination and then die.

Viridis

The seedlings are dark green in the early stages of development and turn normal green in the later stages. The mutants produce normal looking flowers and also set seeds.

Xantha

Colors of the mutants vary from deep yellow to yellowish white. Growth of mutants is retarded and most of them die within 17 to 20 days after emergence.

Chlorina

Normally chlorine mutants do not survive. These mutant seedlings have light yellowish/ yellowish green leaves and culm with yellowish cobs. The mutants breed true for the altered characters.

Effectiveness and efficiency

Mutagenic effectiveness

The sodium azide concentration increased with decreased in the germination per centage. In the treatment 50 per cent lethality was observed at 40mM of sodium azide. The mutagenic effectiveness and efficiency for based on the germination and effectiveness and efficiency observed at on the (Table-3). EMS was found to be more effective than DES and sodium azide in inducing mutation. The maximum mutagenic effectiveness was observed at 50mM of EMS (12.38), while the minimum mutagenic effectiveness was observed at 50mM of sodium azide (6.25).

In mutagenic effectiveness and efficiency was estimated on the basis of relation propagation of families segregation chlorophyll and viable mutants. In comparing EMS, DES and Sodium azide mutagens reduction in plant survival and plant height were observed. In EMS was found to be more effective and efficiency than DES and sodium azide treatment. While the mutagenic effectiveness and efficiency was generally decreased with increase in the higher concentration of mutagens up to certain level. Similar results were recorded by Solanki (2005) in lentil; Sassikumar *et al*. (2003) in Lima bean, Thilagavathi and Mullainathan, (2009) in blackgram.

Mutagenic efficiency

The mutagenic efficiency was worked out based on injury and lethality. On the basis of lethality, the highest mutagenic efficiency was recorded at 50mM of EMS (39.46), while the lowest mutagenic efficiency was observed at 50mM of sodium azide (15.99). In general the mutagenic treatment 50mM of EMS was found to be highly efficient for induced chlorophyll and viable mutants. On the basis of injury, the maximum mutagenic efficiency was observed at 50mM of EMS (70.04). The minimum mutagenic efficiency was observed at 50mM of sodium azide (33.25).Observed in (Table-3).

The mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effect such as injury, lethality and sterility induced by the mutagen (Konzak *et al*., 1965). The EMS is highly efficient than DES and sodium azide. Similar results were recorded by Deepalakshmi and Anandakumar (2003) and Sharma *et al.* (2005) in urdbean; Jayakumar and Selvaraj (2003) in sunflower, Yadava *et al.* (2003) in kodo-millet; Jabee and Ansari (2005) in chickpea; Girija and Dhanavel (2009) in cowpea.

Conclusion

In the present study revealed that the effectiveness and efficiency of EMS, DES and sodium azide. Among these chemical mutagens, EMS was more effective particularly in 50 mM concentration inducing germination, chlorophyll and viable mutants than DES and sodium azide. Therefore chemical mutagens induced the germination, morphological, chlorophyll, effectiveness and efficiency, mutants and desirable agronomical traits which may be possibly utilized in future mutation breeding program.

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Table-1. Effect of chemical mutagenesis in EMS, DES and Sodium azide of LD_{50} values

Treatment (Conc. mM)	Seed germination (%)			Percent over control			Percent of reduction over control		
	EMS	DES	Sodium azide	EMS	DES	Sodium azide	EMS	DES	Sodium azide
Control	93.33	97.33	98.66	100.00	100.00	100.00	00.00	00.00	00.00
10	92.00	92.00	92.00	98.57	94.52	93.24	-1.42	-5.48	-6.76
20	89.33	77.33	76.00	97.71	79.45	77.03	-4.28	-20.55	-22.97
30	76.00	65.33	61.33	81.43	67.12	62.16	-18.56	-32.88	-37.84
40	66.33	49.33	50.66	71.07	50.68	51.34	-28.92	-49.32	-48.66
50	50.66	41.33	40.0	53.96	42.46	40.56	-46.07	-57.57	-59.44
60	40.0	33.33	34.66	42.85	34.24	35.13	-57.14	-65.76	-64.87
70	37.33	25.33	26.66	39.99	26.02	27.02	-60.01	-73.98	-72.98
80	26.66	16.0	21.33	28.56	16.43	21.61	-71.43	-83.57	-78.39
90	13.33	8.0	12.0	14.28	8.21	12.16	-85.71	-91.79	-87.84
100	2.66	2.06	4.0	2.85	2.73	4.05	-97.14	-97.27	-95.95

Table-2. Frequency of chlorophyll and Viable mutants in maize

Mutagens (Conc. mM)	EMS (mM)			DES (mM)			SA (mM)		
	40	50	60	30	40	50	30	40	50
No. of plants studied	137	140	135	141	138	136	140	142	139
Chlorophyll mutants									
Albino	1	2	1	-	1	1	1	1	1
Viridis	2	1	1	1	2	1	-	2	-
Xantha	1	2	2	1	1	1	-	1	1
Chlorina	1	1	1	1	2	2	1	1	1
Viable mutants									
Tall	1	2	1	-	1	-	1	-	2
Dwarf	2	2	2	1	1	1	1	2	-
Early maturity	1	1	1	1	1	1	1	1	1
Late maturity	1	4	2	1	1	-	-	1	1
Triangular leaf	1	2	2	2	1	1	1	2	2
Long ear	1	2	1	2	2	2	1	1	-
Short ear	2	4	2	1	1	1	1	2	2
Male sterility	2	2	1	-	1	1	1	1	1
Total	17	26	19	12	16	14	10	15	13
Frequency	12.40	18.57	14.07	8.51	11.59	10.29	7.14	10.56	9.35

Table 3. Mutagenic Effectiveness and efficiency in M_2 generation

Treatment (Conc. mM)		Survival Reduction (L) 30th day	Height Reduction (I) 30th day	Mutation Frequency	Effectiveness $\frac{M \times 100}{C \times T}$	Efficiency $\frac{M \times 100}{L}$	Efficiency $\frac{M \times 100}{I}$
EMS (Conc. mM)	40	26.68	21.94	12.40	10.33	46.47	56.51
	50	47.05	26.51	18.57	12.38	39.46	70.04
	60	58.04	33.14	14.07	7.81	24.24	42.45
DES (Conc. mM)	30	32.87	17.80	8.51	9.45	25.88	47.80
	40	51.07	22.46	11.59	9.65	22.69	51.60
	50	64.29	30.21	10.29	6.86	16.00	34.06
Sodium azide (Conc. mM)	30	31.42	14.89	7.14	7.93	22.72	47.95
	40	49.29	20.08	10.56	8.80	21.42	52.58
	50	58.46	28.12	9.35	6.25	15.99	33.25