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-1001 which 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 LD50 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.

Materials and Methods

Two sets containing 200 well filled healthy seeds were selected for treatment. To determine the LD50 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, LD50 value was determined. Three concentrations of EMS, DES and Sodium azide around LD50 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...
placed in the moist germination paper and replicated twice for the purpose of laboratory analysis. The seeds harvested from M1 generation were taken from individual treatments and were used to rise M2 generation (Panda and Subuthi, 1994). The M2 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 = Mx100/CxT

Mutagenic efficiency (Lethal) = M x 100 / L

Mutagenic efficiency (Injury) = M x 100 / I

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 M1 generation. In present study the germination, spectrum of chlorophyll in effectiveness and efficiency and total mutations in M2 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 M1 generations.

Seed germination

The germination seeds were counted from 3rd to 7th day recorded. The number of seeds germinated recorded in 15th 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 LD50 values were calculated on the basis of 50 per cent reduction of germination seeds count on 10th day. The germination percentage of Zea mays decreased with increase in the concentration of the mutagens was used to find out the LD50 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 M1 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 M1 and M2 generation. Similar results were observed by soybean (Kavithamani et al., 2008).

Frequency of chlorophyll mutations

The chlorophyll mutations were observed in the M2 generation were scored at the seedling in the field and expressed on M1 panicle family basis as well as on M2 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 M2 plant basis is On M2 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 M2 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, xandha and viridis 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.

Acknowledgement

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References

### Table-1. Effect of chemical mutagenesis in EMS, DES and Sodium azide of LD₅₀ values

<table>
<thead>
<tr>
<th>Treatment (Conc. mM)</th>
<th>EMS (Conc. mM)</th>
<th>DES (Conc. mM)</th>
<th>Sodium azide (Conc. mM)</th>
<th>Seed germination (%)</th>
<th>Percent over control</th>
<th>Percent of reduction over control</th>
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<td>100.00 100.00 100.00</td>
<td>0.00 0.00 0.00</td>
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### Table-2. Frequency of chlorophyll and Viable mutants in maize

<table>
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<tr>
<th>Mutagens (Conc. mM)</th>
<th>EMS (mM)</th>
<th>DES (mM)</th>
<th>SA (mM)</th>
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<tr>
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<td>140</td>
<td>135</td>
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</tr>
<tr>
<td>Albino</td>
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<tr>
<td>Viridis</td>
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</tr>
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<td>Xantha</td>
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<td>2</td>
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<tr>
<td>Chlorina</td>
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<td>1</td>
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<tr>
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<td>Tall</td>
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</tr>
<tr>
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</tr>
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<td>18.57</td>
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### Table-3. Mutagenic Effectiveness and efficiency in M₂ generation

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<tr>
<th>Treatment (Conc. mM)</th>
<th>Survival Reduction (L) 30th day</th>
<th>Height Reduction (I) 30th day</th>
<th>Mutation Frequency</th>
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