COMPARATIVE EFFECTIVENESS AND EFFICIENCY OF PHYSICAL AND CHEMICAL MUTAGENS IN CHICKPEA (CICER ARIETINUM L.)

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ABSTRACT
Mutagenic effectiveness and efficiency of gamma-rays and EMS (Ethyl methane sulphonate) in two cultivars of chickpea (Cicer arietinum L.) viz., HPG-17 (bold seeded) and Himachal chana-1 (small seeded), have been worked out to identify the useful mutagens as well as doses/concentrations. 30kR dose of gamma-rays has been found most effective and efficient on M2 family and M2 plant basis in HPG-17. Among different doses, 0.15% dose of EMS was found more effective on M2 family basis and 0.30% dose was found effective on M2 plant basis and 0.30% dose of EMS was found efficient in HPG-17. In Himachal chana-1, 30kR dose was most effective and 50kR dose of gamma-rays was found to be most efficient and among different doses of EMS, 0.15% dose was observed as the most effective and 0.30% dose as most efficient. In general, EMS was found to be more effective and gamma-rays more efficient in bold seeded variety, whereas in small seeded variety, EMS was found to be more effective and efficient. Combined analysis of mutagens and varieties showed that chemical mutagen (EMS) was more effective and efficient than physical mutagen (gamma-rays) in inducing mutations and mutation rate was more in small seeded variety than in bold seeded variety.

INTRODUCTION
In leguminous crops such as chickpea, improved cultivars are developed through conventional breeding methods. A critical appraisal of all the chickpea breeding work in the country and at global scale indicates that like other grain legumes, little concentrated efforts have been made for its genetic amelioration. The hybridization work undertaken is also very limited and it is probably due to the tedious crossing procedure because of small and delicate flowers. Induced mutagenesis can play role in renewal as well as augmentation of the natural variability to some extent.

Although extensive studies on induced variations in cereal crops have been undertaken in the past, but very few reports on comparative effectiveness and efficiency of different physical and chemical mutagens are available for chickpea (Srivastava, et al. 1973, Kharkwal, et al. 1986, Kharkwal, 1998), which is covering more than 50% area among all pulse crops. Before going for genetic improvement of a crop through mutation breeding, a thorough knowledge of mutagenic effectiveness and efficiency of mutagens to be used is essential to identify the useful mutagens as well as doses/concentrations for effective breeding programme. Efficient mutagenesis means the production of maximum desirable changes accompanied by the least possible undesirable changes. Mutagenic effectiveness is a measure of the frequency of mutations induced per unit dose of mutagen. Mutagenic efficiency is indicative of the proportion of mutations as against associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility, induced by the mutagen in question. The present studies were undertaken to find out the effectiveness, efficiency of physical and chemical mutagens in two desi cultivars of chickpea.

MATERIAL AND METHODS
The material used in the study comprised of two varieties of chickpea (Cicer arietinum L.), HPG-17 (bold seeded) and Himachal chana-1 (small seeded). The treatment with gamma rays was given with 60Co gamma cell delivering 1800 rad per minute. Three doses of gamma rays (30kR, 40k, 50kR) were given to each variety. In case of EMS, three
concentrations (0.15%, 0.30% and 0.45%) were used but no germination in M1 occurred at 0.45% in case of HPG-17, observations were confined only in two concentrations (0.15%, 0.30%) in case of HPG-17. The treatments with EMS were given for three hrs in phosphate buffer (pH 7) with intermittent shaking. Dry seed were used as control. The seeds were soaked in distilled water for 16 hrs before treatment and were washed thoroughly under running tap water for 30 minutes to remove residual chemical and then dried on blotting paper. Treated and control seeds were sown in rows of 3m length with 15cm and 45 cm plant to plant and row to row spacing, respectively on the same day in the field. Each M1 plant was harvested separately and M2 progenies were raised in separate rows. Chlorophyll and viable mutations were scored in the field for entire cropping season. Mutagenic effectiveness and efficiency were worked out using following formula (Konzak et al. 1965):

**Mutagenic effectiveness :**

\[
\text{Mutation rate (M}_2\text{ family or plant basis)} = \frac{\text{Time in hrs.} \times \text{Conc.}}{\text{Dose in kilo roentgen}}
\]

**Mutagenic efficiency :**

\[
\text{Mutation rate (M}_2\text{ family or plant basis)} = \frac{\text{Percentage of biological injury}}{\text{Number of mutated M}_2\text{ families}} \times 100
\]

**Mutation frequency on M}_2\text{ family basis : (% of mutated M}_2\text{ families)}

\[
\frac{\text{Total M}_2\text{ families}}{\text{Number of mutated families}} \times 100
\]

**Mutation frequency on M}_2\text{ plant basis : (% of mutated M}_2\text{ plants)}

\[
\frac{\text{Total M}_2\text{ plants}}{\text{Number of mutated plants}} \times 100
\]

RESULTS AND DISCUSSION

**Mutagenic Effectiveness :** The data presented in Table 1 indicate that the effectiveness of the mutagens, worked out on the basis of M1 family basis and M2 plant basis and response of the varieties were varying. The effectiveness of different doses of gamma-rays and EMS in bold seeded variety (HPG-17) revealed that among different doses of gamma-rays, 30kR was most effective both on M1 family basis and M1 plant basis. Among different doses of chemical mutagen, EMS @ 0.15% was found to be more effective on M1 family basis and 0.30% dose was found to be more effective on M1 plant basis. In small seeded variety (Himachal chana-1) 30kR dose of gamma-rays and 0.15% dose of EMS, were observed to be the most effective on mutant family and plant basis. Comparative effectiveness of different doses of gamma-rays and EMS in bold seeded variety revealed 0.15% dose of EMS as the most effective dose on mutant family basis and 0.30% as the most effective dose on mutant plant basis (Fig. 1). However in small seeded variety 0.15% dose of EMS was found to be the most effective dose on mutant family and plant basis among different doses of physical and chemical mutagens (Fig. 2). When treatments were pooled over varieties, it was found that among different doses of physical mutagen (gamma-rays), 30kR dose was found most effective on M1 family basis and 30kR dose was at par with 40kR dose in effectiveness on M1 plant basis and 0.15% dose of EMS was most effective both on M1 family and plant basis (Table 2). Overall, 0.15% dose of EMS was observed to be the most effective among different doses (Fig. 3.). On the basis of mutagens pooled over varieties, EMS was found to be more effective than gamma-rays. The response of varieties and effectiveness of mutagens was observed to be different. Both physical and chemical mutagens showed inverse relationship for dose dependency.

**Mutagenic Efficiency :** Mutagenic efficiency was also worked out on M1 family and M1 plant basis (Table 1). In HPG-17, 30kR dose of gamma-rays and 0.30% dose of EMS was observed to be most efficient on mutant family and plant basis. While working out the
Fig. 1. Comparative effectiveness of gamma-rays and EMS in HPG-17.

Fig. 2. Comparative effectiveness of gamma-rays and EMS in Himachal chana-1.

Fig. 3. Comparative effectiveness of treatments over pooled varieties.

Fig. 4. Comparative effectiveness of mutagens pooled over varieties.

Fig. 5. Comparative effectiveness of gamma-rays and EMS in HPG-17.

Fig. 6. Comparative efficiency of gamma-rays and EMS in Himachal chana-1.
comparative efficacy of different doses of both the mutagens, it was observed that 30kR dose of gamma-rays was the most efficient one (Fig. 4). In Himachal chana-1, 50kR dose of gamma-rays and 0.30% dose of EMS showed highest efficiency over all doses both on mutant family and mutant plant basis. In small seeded variety 0.30% dose of EMS was observed to be the most efficient, when comparative efficacy of different doses of gamma-rays and EMS were compared (Fig. 5). When comparative efficiency of different doses of gamma-rays and EMS was worked out by pooling varieties, 50kR dose among different doses of gamma-rays and 0.30% dose among different doses of EMS, were found to be highly efficient both on M2 family and M2 plant basis. Pooled analysis of mutagens over varieties revealed higher efficiency of EMS over gamma-rays on M2 family and M2 plant basis (Table 2). Comparative efficiency of treatments pooled over varieties, revealed 0.30% dose of EMS as the most efficient dose (Fig. 6).

The average mutagenic effectiveness was higher at lower doses of gamma-rays and EMS. This may be due to the fact that saturation point (dose/concentration at which all mutable loci get mutated) occur at

### TABLE 1. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%seedling height reduction</th>
<th>Mutation rate</th>
<th>Mutagenic effectiveness</th>
<th>Mutagenic efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Family basis</td>
<td>Mutant family basis</td>
<td>Family plant basis</td>
</tr>
<tr>
<td>HPG-17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma-rays</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30kR</td>
<td>9.16</td>
<td>9.52</td>
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<td>40kR</td>
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<td>11.54</td>
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<tr>
<td>50kR</td>
<td>19.45</td>
<td>5.56</td>
<td>0.57</td>
<td>0.11</td>
</tr>
<tr>
<td>Average</td>
<td>22.08</td>
<td>8.87</td>
<td>0.92</td>
<td>0.24</td>
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<tr>
<td>EMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15%</td>
<td>17.50</td>
<td>4.76</td>
<td>0.68</td>
<td>1.09</td>
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<td>0.30%</td>
<td>23.39</td>
<td>7.14</td>
<td>1.89</td>
<td>0.82</td>
</tr>
<tr>
<td>Average</td>
<td>20.45</td>
<td>5.95</td>
<td>1.29</td>
<td>0.96</td>
</tr>
<tr>
<td>Himachal chana-1</td>
<td></td>
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<td>Gamma-rays</td>
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</tr>
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<td>Average</td>
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<td>14.22</td>
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<td>0.36</td>
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<tr>
<td>EMS</td>
<td></td>
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</tr>
<tr>
<td>0.15%</td>
<td>1.54</td>
<td>22.86</td>
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</tr>
<tr>
<td>0.30%</td>
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<tr>
<td>Average</td>
<td>14.84</td>
<td>19.79</td>
<td>2.27</td>
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lower doses of mutagens and further increase in
dose does not result in any change in mutation
rate. Higher effectiveness and efficiency of
physical (gamma-rays) and chemical mutagens
(MMS, DES, EMS and NMU) at lower doses
has been also reported in peas (Gad and El-
Sawah, 1985) and in lentil (Solanki and Sharma,
1994).

In bold seeded variety HPG-17
lowest dose and in small seeded variety highest
dose of gamma-rays was found most efficient.
It clearly indicated that in bold seeded variety
mutation frequency increases while damage
remains constant or decreases at higher doses,
whereas in small seeded variety the frequency
of desirable mutations increased with increase
in dose of gamma-rays. In small seeded variety
(Himachal chana-1) highest dose was found
most efficient, meaning thereby that
rate of undesirable mutations was more than
desirable ones at 0.45% dose of EMS. EMS
was found to be more effective and gamma-
rays more efficient in bold seeded variety,
whereas in small seed variety, EMS was
found to be more effective and efficient. This
may be due to the fact that the most effective
agent or treatment may not be the most
efficient. The apparent mutagenic efficiency as
well as effectiveness of any two agents may
differ with genotypes because the comparable
test conditions may not permit the expression
of the true potential of the agents. Efficient
treatments are essential for economical use of
mutagens as tools for direct improvement, or
for the induction of certain qualitative or
quantitative traits.

Chemical mutagen (EMS) was
more effective and efficient than physical
mutagen (gamma-rays) in inducing mutations.
This may be due to the fact that chemical
mutagens are less drastic in their effects than
ionizing radiations, producing more gene
mutations and fewer chromosome disruptions,
secondly action of chemical mutagens may be
delayed so that the mutation does not appear
until several cell divisions have occurred. EMS
has been also reported as the most effective
and efficient mutagen in comparison to DES
and gamma-rays in rice (Kaul and Bhan, 1977).
Chemical mutagens are not only more effective

<table>
<thead>
<tr>
<th>Treatment basis*</th>
<th>Mutagen basis#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutagen</td>
<td>Treatment Effectiveness</td>
</tr>
<tr>
<td></td>
<td>Family Mutant</td>
</tr>
<tr>
<td>Gamma rays</td>
<td>30kR</td>
</tr>
<tr>
<td></td>
<td>40kR</td>
</tr>
<tr>
<td></td>
<td>50kR</td>
</tr>
<tr>
<td>EMS</td>
<td>0.15%</td>
</tr>
<tr>
<td></td>
<td>0.30%</td>
</tr>
<tr>
<td></td>
<td>0.45%</td>
</tr>
</tbody>
</table>

* Treatments pooled over varieties; # Mutagens pooled over varieties and treatments.
but also more efficient than physical mutagens in inducing viable and total number of mutations in chickpea (Kharkwal, 1999). Similarly, in grasspea (Lathyrus sativus L.), higher effectiveness and efficiency of EMS than gamma-rays were reported (Waghmare and Mehra, 2001).

Despite of the fact that highest mutation rates were, in general, obtained even with higher doses/ concentrations both in case of gamma-rays and EMS, both mutagenic effectiveness and efficiency generally decreased with the increase in dose/ concentration of mutagens with some exceptions. From this it is clear that higher mutagenic effectiveness and efficiency dose not reflect the per se mutation frequency, hence can not be used as an index for maximization of mutation rates.

**REFERENCES**


