Study of the Impact of EMS-induced Mutations on the Growth and Morpho-Phenological Traits in Pea (*Pisum sativum* L)

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**ABSTRACT**

This study was intended to compare the effectiveness and the efficiency of ethyl methane sulfonate (EMS) for inducing mutation in pea seeds by considering various morphological and agronomical characters such as the onset of germination, plant height, number of tendrils, days for flowering and branches at maturity. Seeds were treated with 0.1% of EMS and compared with control seeds. The M1 generation has produced from the mutagen treated seeds. Thus, the material has shown great variation with respect to morphological and agronomical characters. Significant results have been observed with respect to germination period: mutant pea seeds took two to fourteen days to germinate while control seed took two to four days to germinate. Also, a significant reduction in the maturity of tendrils and their respective heights was observed after 18 days of mutant germination. Non-significant results were observed with respect to number of days taken for flowering and branches at maturity as compared to control. Significant reduction in height has been observed at maturity in mutant as compared to control.

**KEYWORDS** pisum sativum, mutation breeding, ethyl methane sulfonate (EMS), induced mutation, morpho-phenological traits, etc.

**INTRODUCTION**

Pea (*Pisum sativum* L.), a commercially important crop for food and feed, belongs to family Fabaceae (formerly Leguminosae), subfamily Papilionoideae. It is a protein rich, self-pollinated (Gill and Vear, 1980) cool season, the plant is a diploid (2n = 14) (Hancock, 2004) and genome size is 5000 mb. The third largest area in pea cultivation is occupied by India, after Canada and Russia. The seeds of pea are rich in 23% protein and 50% digestible starch. During 2000 to 2010 about 6 - 6.5 million/ha dry seeds were cultivated. The induced mutations offer the possibility for the induction of desired changes in various attributes, which can be exploited as such or through recombination breeding (Cheema & Atta, 2003; Khin, 2006).

Mutation breeding is one of the conventional method of plant breeding to improve crops by using mutational agents such as physical and chemical. Breeding efforts have been made with pea to develop high-yielding variety in the last decades using local or introduced material in Turkey, (Bilgili and Acikgoz, 1999; Sayar et al., 2009). Until now, a variety development study on pea landraces of the northern part of the Eastern Anatolia region has not been recorded.

Mutational breeding is used to induce mutations in loci to control economically the important traits or eliminate undesirable genes from elite breeding lines (Lippert et al., 1964). Mutations may arise spontaneously or may be induced. Mutations can be induced by using radioactive or chemical mutagens. In chemical mutagens like ethyl methane sulfonate (EMS) and DMS, MMS, radiation and transposons are used to generate mutants. EMS induces a vastly higher proportion of point mutations (Minocha and Arnason, 1962). In plants, EMS usually causes point mutations, but the loss of a chromosome segment or deletion can also occur. Mutations in seeds of spring rape were induced artificially (Thurling and Depittayanan, 1992), herbicide tolerance in soybean (Sebastian et al., 1989), male sterility in wheat (Maan and Williams, 1984). The high efficiency of EMS for creating phenotypic variation like potato shaped leaves, reduced fruit size, and maximum disease resistance were observed in tomato (Yudhvir, 1995).

Dhulgande et al. (2015) tried different concentration of EMS (0.05%, 0.10%, 0.15% and 0.20%) for studying seed germination, seedling height, (shoot and root), seedling injury, seedling vigour index, and seedling survival of plants at 30th day. The seed germination percentage was decreased with increase in the concentration/doses when compared to control. The decrease in seed germination was more prominent with EMS treatments. The seedling parameter EMS treated seedlings were progressively
decreased with an increase in dose/concentration in all mutagenic treatments when compared to control. Comparatively, maximum seedling parameters were observed in EMS concentrations.

Konzak et al. (1965) while working on two varieties of pea DDR-53 and DMR-55 stated that usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness and chemical mutagen (EMS). The treatments included three doses of gamma rays (5kR, 7kR, and 10kR) and three concentrations of EMS (0.05%, 0.10% and 0.15%) and observed the variation in pea. Mutation breeding causes variation in the phenotypic and morphological character of the plant. Different parameters are used for phenotypic characterization. Like days of flowering, days of harvesting, the height of the plant, pod length, pod per plant, test weight, seed per plant, leaf size, nature of tendril, number of pods, root type, the distance between two nodes, vein pattern, number of branches, etc. These parameters used for observing variation occur in the mutated plant.

Jabeen and Mirza (2004) reported that the induced morphological mutations in Capsicum annuum cultivar Longhi among chemical mutagens, the alkylating agent, EMS is the most commonly used in plants as it causes a high frequency of nucleotide substitutions, as detected in different genomes. The seeds of potential genotype of the popular variety, seeds were treated with EMS at concentrations of 0.1%, 25%, 0.50%, 0.75%, 1%, 1.25%, 1.5% and 2%. Sensitivity to EMS was determined by various measurements on the M1 generation. As the concentration of applied EMS increased the decrease in germination, seedling height, root length and emergence under field conditions were observed in M1 generation as compared to the non-treatment control.

### MATERIALS AND METHODS

**Plant material:**

The plant material was taken from a wild variety of pea (P. sativum L.). The 1400 seeds of wild pea varieties were used for this work. EMS treatments were given to 1200 healthy seeds, and the remaining 200 seeds were used as control seeds.

**Preparation of 0.1% EMS solution:**

0.1% of EMS solution was prepared by taking 1 ml (EMS). This solution was added to 999 ml of distilled water.

**Seed treatment with EMS:**

EMS treatment was very effective in pea plant (P. sativum L.), 1200 healthy selected seeds of wild Pea plant were taken in a conical flask with 1000 ml distilled water, 1 ml EMS was added in the conical flask while the remaining 200 controlled seeds were left untreated. Now, the 1200 selected seeds were treated with 0.1% (EMS) at 25°C by keeping on a rotary shaker for 16 hours. Treated seeds were washed with tap water for 4 hours continuously. The remaining 0.1% of EMS solution was washed out by using 10% Sodium thiosulphate solution and kept at room temperature for 24 hours. Washing of seeds was done in running tap water for 4 hours to remove residual EMS from the surface of the seeds and then kept for drying on the filter paper at room temperature for two hours for the removal of excess moisture before sowing. After drying, both control and treated seeds were sown in the plastic pots according to random block design (RBD). The spacing between two pots was kept around two feet. Statistical analysis was done and observation was recorded for the following characters as listed in Table 1.

### RESULTS AND DISCUSSION

Effectiveness and efficiency of EMS for inducing mutation in pea seeds were evaluated by considering various morphological and agronomical characters such as the onset of germination, plant height, number of tendrils, days for flowering and branches at maturity.

1. **Onset of germination (T1)**

At the onset of germination, mutant seeds (1200) took more days for germination as compared to the control seeds (200). Mutant seeds took four days for their germination and control seeds took two days. Thus, EMS affects the germination of pea (P. sativum) in mutant seeds such that their complete germination took 2–14 days but control seeds took maximum 2–4 days. Mean value for mutant was 4.267 while the mean value of control seeds was 2.325. Extreme value for mutant is 14 as shown in Table 2 and Fig 1 to shows the difference between control and mutant seeds.

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T1 – Onset of germination</td>
</tr>
<tr>
<td>2</td>
<td>T2 – Plant height at 18 DAS</td>
</tr>
<tr>
<td>3</td>
<td>T3 – Plant height at maturity</td>
</tr>
<tr>
<td>4</td>
<td>T4 – Number of tendrils 18 DAS</td>
</tr>
<tr>
<td>5</td>
<td>T5 – Branches at maturity</td>
</tr>
<tr>
<td>6</td>
<td>T6 – Days of flowering</td>
</tr>
</tbody>
</table>

| Table 1: Characters of P. sativum. |
Impact of EMS-induced mutations in pea

Table 2: To study the impact of EMS-Induced mutation on the germination.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Mean</th>
<th>Median</th>
<th>Mode</th>
<th>C.V</th>
<th>Variance</th>
<th>Kurtosis</th>
<th>Shapiro–Wilk normality test (P)</th>
<th>Wilcoxon rank sum test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.325</td>
<td>2.000</td>
<td>2.000</td>
<td>0.526</td>
<td>22.608</td>
<td>0.276</td>
<td>2.2e-16</td>
<td>Non normal</td>
</tr>
<tr>
<td>Mutant</td>
<td>4.267</td>
<td>4.000</td>
<td>3.000</td>
<td>1.949</td>
<td>45.670</td>
<td>3.798</td>
<td>5.428e-09</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Fig. 1 Impact of EMS-induced mutation of the germination.

Table 3: To study the impact of EMS-induced mutation on the plant heights.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Mean</th>
<th>Median</th>
<th>Mode</th>
<th>S.D</th>
<th>C.V</th>
<th>Variance</th>
<th>Kurtosis</th>
<th>Shapiro–Wilk normality test (P)</th>
<th>Wilcoxon rank sum test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.363</td>
<td>14.000</td>
<td>13.000</td>
<td>2.665</td>
<td>18.55</td>
<td>7.102</td>
<td>-0.649</td>
<td>0.4005</td>
<td>Normal</td>
</tr>
<tr>
<td>Mutant</td>
<td>9.702</td>
<td>10.000</td>
<td>10.000</td>
<td>2.949</td>
<td>30.39</td>
<td>8.696</td>
<td>0.393</td>
<td>3.781e-07</td>
<td>Not normal</td>
</tr>
</tbody>
</table>

2. Plant height at 18 DAS (T2)
Compared to the control seeds plant height; a significant reduction was observed at 18 days in mutant seeds after sowing. The mean value and extreme value of mutant seeds were 9.702 and 23, respectively. The mean value of control was 14.363 as mentioned in Table 3 and Fig. 2.

3. Plant height at maturity (T3)
Significant reduction in height was observed at maturity in mutant as compared to control. The mean value of mutant was 33.635 and extreme value in case of the mutant was 60. The mean value of control 39.225 is depicted in Table 4 and Fig. 3 shows the variation between control and mutant seed.

4. Numbers of tendrils 18 DAS (T4)
The significant reduction in number of tendrils in the mutant was observed as compare to control at 18 days after sowing. The mean value of mutant was 7.188 and extreme value in case of the mutant was 18. The mean value of control 8.675 was observed and presented in Table 4 and Fig. 4 shows the variation of control and mutant seeds.

5. Days of flowering (T5)
In this the days of flowering in mutant and control were non-significant (Table 6). The mean value of mutant was 46.668, but the extreme value was 72. The extreme value of mutant is much higher than control. The mean value of control was 43.9 and Fig. 5 observed the variation in flowering.

6. Branches at maturity (T6)
In this experiment, the difference between numbers of branches in mutant and control was non-significant at maturity (Table 7). The mean value of mutant was 1.535,
Amol Ramesh Savant

Table 4: To study the impact of EMS-induced mutation on the plant heights at maturity.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Mean</th>
<th>Median</th>
<th>Mode</th>
<th>S.D</th>
<th>C.V</th>
<th>Variance</th>
<th>Kurtosis</th>
<th>Shapiro–Wilk normality test (P)</th>
<th>Wilcoxon rank sum test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.225</td>
<td>38.000</td>
<td>38.000</td>
<td>5.780</td>
<td>14.736</td>
<td>33.410</td>
<td>0.478</td>
<td>0.08007 Normal</td>
<td>1.65e-05</td>
</tr>
<tr>
<td>Mutant</td>
<td>33.635</td>
<td>35.000</td>
<td>38.000</td>
<td>7.733</td>
<td>22.991</td>
<td>59.802</td>
<td>0.751</td>
<td>8.141e-08 Non normal</td>
<td>Significant</td>
</tr>
</tbody>
</table>

**Table 5:** To study the impact of EMS-induced mutation on the number of tendrils.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Mean</th>
<th>Median</th>
<th>Mode</th>
<th>S.D</th>
<th>C.V</th>
<th>Variance</th>
<th>Kurtosis</th>
<th>Shapiro–Wilk normality test (P)</th>
<th>Wilcoxon rank sum test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.675</td>
<td>8.000</td>
<td>7.000</td>
<td>2.536</td>
<td>39.231</td>
<td>6.430</td>
<td>7.944</td>
<td>3.822e-08 Non normal</td>
<td>9.72e-06</td>
</tr>
<tr>
<td>Mutant</td>
<td>7.188</td>
<td>7.000</td>
<td>7.000</td>
<td>1.927</td>
<td>26.814</td>
<td>3.714</td>
<td>3.406</td>
<td>2.589e-16 Non normal</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Fig. 3: Impact of EMS-induced mutation on the plant heights at maturity.

Fig. 4: Impact of EMS-induced mutation on the numbers of tendrils, 18 DAS.

but the extreme value was much higher; it was 8 (Fig. 6). The mean value of control was 1.325. It was similar to the observation of 18 days after sowing.

**DISCUSSION**

Present investigation showed the impact of EMS-induced mutation on the germination of pea seeds. The early seed germination was observed in control than mutated seeds; this indicates the effect EMS (mutagen) on germination of seeds. Dhulgande et al. (2015) also reported the percentage of seed germination and seedling growth inhibited with an increasing dose/concentration of mutagens. The survival rate was highly reduced with an increasing dose/concentration of mutagens. The significant reduction was observed in plant height at 18 days after sowing. Mustafa et al. (2012) observed the variation of the height that is 83.5–126 cm and at maturity in mutant as compare to control. The numbers of tendrils were affected by the mutation. Numbers of tendrils were decreased in mutant plants. The days of flowering in mutant and control were non-significant. The numbers of branches were same in control as well as in mutant at 18 DAS, but Wiltshire et al. (1994); Villani and DeMason (2000) reported that after 30 DAS the number of branches were not much increased in the mutant, but in some mutant plant drastic branching were observed. Generally, all control showed no branching or single branching.

**CONCLUSION**

The impact of EMS-induced mutation on the growth and morpho-phenological traits in pea (*P. sativum*) were analysed. The percentage of seed germination and seedling growth were inhibited with an increasing concentration of mutagen. The days taken for flowering and branches at maturity parameters were observed to be non-significant with an increasing concentration of mutagen. Almost all the mutagenic treatments showed significant results with respect to germination period, reduction in the maturity of tendrils and their respective
Table 6: To study the impact of EMS-induced mutation on the days of flowering.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Mean</th>
<th>Median</th>
<th>Mode</th>
<th>S.D</th>
<th>C.V</th>
<th>Variance</th>
<th>Kurtosis</th>
<th>Shapiro–Wilk normality test (P)</th>
<th>Wilcoxon rank sum test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43.950</td>
<td>42.500</td>
<td>42.000</td>
<td>2.935</td>
<td>6.677</td>
<td>8.613</td>
<td>3.903</td>
<td>5.593e-07 Non Normal</td>
<td>3.588e-07</td>
</tr>
<tr>
<td>Mutant</td>
<td>46.668</td>
<td>46.000</td>
<td>48.000</td>
<td>4.203</td>
<td>9.006</td>
<td>17.666</td>
<td>4.943</td>
<td>2.2e-16 Non normal</td>
<td>Non significant</td>
</tr>
</tbody>
</table>

Fig. 5 Impact of EMS-induced mutation on the days of flowering.

Table 7: To study the impact of EMS on branches at maturity.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Mean</th>
<th>Median</th>
<th>Mode</th>
<th>S.D</th>
<th>C.V</th>
<th>Variance</th>
<th>Kurtosis</th>
<th>Shapiro–Wilk normality test (P)</th>
<th>Wilcoxon rank sum test (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.325</td>
<td>1.000</td>
<td>1.000</td>
<td>0.616</td>
<td>46.453</td>
<td>0.379</td>
<td>7.925</td>
<td>8.503e-10 Non Normal</td>
<td>0.08753</td>
</tr>
<tr>
<td>Mutant</td>
<td>1.524</td>
<td>1.000</td>
<td>1.000</td>
<td>0.760</td>
<td>49.894</td>
<td>0.578</td>
<td>6.420</td>
<td>2.2e-16 Non normal</td>
<td>Non-significant</td>
</tr>
</tbody>
</table>

Fig. 6 Impact of EMS-induced mutation on the branches at maturity.

heights at laboratory condition. The chemical mutagen, showed more positive effect towards most of the parameters as compared to control.

REFERENCES


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