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# MUTATION STUDIES IN CHICKPEA *(CICER ARIETINUM* L.) 1. MUTAGEN SENSITIVITY

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Three kabuli chickpea genotypes, viz. ILC's 482, 3279 and 6104 were treated with a range of gamma irradiation doses (10 to 110 kR) and EMS doses (0.1 to 0.4%) to obtain useful information about the use of these mutagens in inducing genetic variability and to estimate the doses of gamma irradiation and EMS that were effective to reduce the growth by a given proportion under control (non-treated) conditions. At lower doses of gamma irradiation, stimulating effect on shoot and root length was observed in all the genotypes but adversely affected at higher doses of gamma irradiation and EMS. The sensitivity to gamma irradiation and EMS appeared to be related with the seed size. The large seeded genotype ILC 6104 seemed to be more sensitive than the small seeded ILC 3279 and ILC 482. It appeared that shoot and root length reduction can be used with equal reliability for estimating the appropriate mutagen dose. Three gamma irradiation doses (40, 50 and 60 kR) and two EMS doses (0.1 and 0.2%) which caused reduction in the shoot and root length around 20-40% could be chosen for large scale mutagenic treatments.

## INTRODUCTION

Mutation breeding has been used in recent years as a valuable supplement to the methods of plant breeding in the development of better crop cultivars. The gamma irradiation treatments have been used more frequently. Of 84 mutant cultivars of food legumes released, 53 have been developed through the use of gamma rays (Micke, 1988). Ethylmethane sulphonate is the most powerful and most recommendable among chemical mutagens for seed treatment because it produces high frequency of mutations accompanied by a relatively low frequency of chromosomal aberrations in plants.

Determination of a suitable radiation dose for a particular cultivar is of primary importance in studies on mutation breeding. Higher doses produce very drastic effect which may lead to death of the organism. Relatively lower doses often results in altered growth characteristics. The term lower and higher are relative and may be different for different systems. Besides there are differences in radiation tolerance among species (Sparrow, 1966) and even among genotypes of the same species. Seed germination, seedling growth and chromosomal aberrations are the commonly used criteria for radiosensitivity in plants and in certain cases $M_1$ sterility has been shown to be appropriate criterion. The estimation of growth reduction dose (GRD) has been discussed by Finney (1971) for binary response. Various forms of dose and binary response relationship have also been reported in literature (Singh, 1987).

This experiment was conducted to obtain useful information about the use of gamma irradiation and EMS in inducing genetic variability in kabuli chickpea genotypes and to estimate the doses of gamma irradiation and EMS, effective to reduce the growth of shoot and root length by a given proportion of the growth under control (non-treated) conditions. For example, GRD 50 would be effective in reducing the growth response by half of the response under control.

## MATERIALS AND METHODS

The seeds of three chickpea genotypes viz. ILC 482 (Origin:Turkey; special feature:bush types growth habit, medium maturity, medium seed size, 29 g hundred seed weight (HSW), tolerant to *Ascochyta rabiei* (Pass) Lab. diseases), ILC 3279 (Origin:USSR; special feature:tall growth habit, late maturity, medium seed size, 28 to 29 g HSW, moderately resistant to *Ascochyta* blight disease) and ILC 6104 (Origin:Mexico; special feature:bush type growth habit, early maturity, large seed size, 55 g HSW, susceptible to *Ascochyta* blight disease) were used in this study. Dry seeds of these genotypes were taken from the same harvest and graded to a uniform size at International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria during May, 1987.

Seeds were then brought to Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan for mutagenic treatment and laboratory experiment. Moisture content in seed at the time of mutagenic treatment was brought to 11% by keeping them in a desiccator containing calcium chloride. Seeds were subjected to gamma irradiation and doses of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 and 110 kR were administered to 40 seeds for each treatment in three genotypes. Gamma irradiation was carried out at NIAB at room temperature (22-25 °C) in Cobalt$^{60}$ gamma cell-220 (Atomic Energy of Canada Ltd.) of 381.43 curie strength delivering 29 kR $hr^{-1}$ at the time of irradiation.

Prior to the chemical treatment, seeds were pre-soaked in distilled water. After one hour of soaking under continuous aeration, the excess water was drained and the swollen seeds were subjected to 0.1, 0.2, 0.3 and 0.4% aqueous solutions of EMS for one hour with constant shaking. The volume of mutagen used was three times more than that of seed volume. Seeds soaked in water were kept as control in this case. After treatment, seeds were washed in running tap water for one hour to leach the residual chemical and then were dried on blotting paper. All these operations were carried out at room temperature.

The treated and non-treated (control) seeds were grown at NIAB same day after treatment in petriplates having sand. The petriplates were placed in an incubator maintained at 25 ± 1 °C. For each treatment 10 seeds were used in each replication.

Data on germination were recorded by counting total number of plants germinated in each treatment. Shoot and primary root length of seedling were recorded in centimeters on 5 randomly selected plants from each treatment on 5th day after seeding.

Data on germination, shoot and root length were subjected to analysis of variance. GRD 30, 40 and 50 were determined on the basis of reduction of shoot and root length.

## RESULTS AND DISCUSSION

Mean germination percentage, shoot length and root length in three chickpea genotypes after treatment with different doses of gamma irradiation and EMS are presented in Table 1.

**Table 1.** Germination and shoot and root length in chickpea genotypes after treatment with different doses of gamma irradiation and ethylmethane sulphonate (EMS) in laboratory experiment

**a. Two-way table of means**

| Treatment | Germination (%) | | | Shoot length (cm) | | | Root length (cm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | ILC 482 | ILC 3279 | ILC 6104 | ILC 482 | ILC 3279 | ILC 6104 | ILC 482 | ILC 3279 | ILC 6104 |
| Cont (Gamma) | 100.0 | 100.0 | 100.0 | 8.850 | 9.100 | 3.275 | 10.700 | 12.575 | 6.60 |
| 10 kR | 100.0 | 97.5 | 100.0 | 9.400 | 8.925 | 4.625 | 11.400 | 12.100 | 8.10 |
| 20 kR | 100.0 | 97.5 | 100.0 | 9.175 | 9.225 | 3.725 | 11.000 | 12.775 | 7.00 |
| 30 kR | 100.0 | 92.5 | 100.0 | 8.700 | 8.900 | 3.550 | 9.700 | 10.250 | 6.77 |
| 40 kR | 100.0 | 90.0 | 100.0 | 7.525 | 7.500 | 3.075 | 8.200 | 9.300 | 5.37 |
| 50 kR | 100.0 | 90.0 | 100.0 | 6.425 | 6.825 | 2.675 | 7.100 | 8.575 | 4.69 |
| 60 kR | 100.0 | 85.0 | 100.0 | 5.850 | 5.950 | 2.070 | 6.200 | 7.650 | 3.81 |
| 70 kR | 97.5 | 82.5 | 95.0 | 4.725 | 4.500 | 1.585 | 5.650 | 5.700 | 2.99 |
| 80 kR | 97.5 | 80.0 | 80.0 | 3.975 | 3.900 | 1.450 | 4.350 | 4.500 | 2.20 |
| 90 kR | 97.5 | 80.0 | 80.0 | 3.500 | 3.095 | 0.700 | 3.900 | 3.900 | 2.00 |
| 100 kR | 95.0 | 75.0 | 80.0 | 3.100 | 2.500 | 0.575 | 3.700 | 2.900 | 1.50 |
| 110 kR | 92.5 | 75.0 | 70.0 | 2.800 | 1.400 | 0.425 | 2.700 | 2.600 | 1.20 |
| Cont (EMS) | 100.0 | 100.0 | 100.0 | 9.000 | 9.450 | 3.480 | 11.700 | 12.750 | 7.77 |
| 0.1% | 100.0 | 100.0 | 80.0 | 7.950 | 8.425 | 2.840 | 9.650 | 9.650 | 5.45 |
| 0.2% | 100.0 | 90.0 | 70.0 | 5.970 | 5.950 | 2.120 | 7.350 | 7.375 | 4.11 |
| 0.3% | 92.5 | 75.0 | 57.5 | 4.550 | 4.675 | 1.620 | 5.700 | 6.575 | 3.76 |
| 0.4% | 80.0 | 60.0 | 50.0 | 3.200 | 2.875 | 0.930 | 3.925 | 4.850 | 2.43 |
| SE 1 | ± 2.85 | | | ± 0.178 | | | ± 0.186 | | |
| SE 2 | ± 2.88 | | | ± 0.174 | | | ± 0.179 | | |

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**b. One-way tables of means**

| | Germination (cm) | Shoot length (cm) | Root length (cm) |
|---|---|---|---|
| Genotype | | | |
| ILC 482 | 97.21 | 6.159 | 7.231 |
| ILC 3279 | 86.47 | 6.070 | 7.884 |
| ILC 6104 | 86.03 | 2.278 | 4.458 |
| SE | ± 0.57 | ± 0.057 | ± 0.068 |
| Mutagen | | | |
| Control (gamma) | 100.00 | 7.075 | 9.958 |
| 10 kR | 99.17 | 7.650 | 10.533 |
| 20 kR | 99.17 | 7.375 | 10.258 |
| 30 kR | 97.50 | 7.050 | 8.907 |
| 40 kR | 96.67 | 6.033 | 7.625 |
| 50 kR | 96.67 | 5.308 | 6.790 |
| 60 kR | 95.00 | 4.623 | 5.888 |
| 70 kR | 91.67 | 3.603 | 4.780 |
| 80 kR | 85.83 | 3.108 | 3.683 |
| 90 kR | 85.83 | 2.432 | 3.267 |
| 100 kR | 83.33 | 2.058 | 2.700 |
| 110 kR | 79.17 | 1.542 | 2.168 |
| Control (EMS) | 100.00 | 7.310 | 10.740 |
| 0.1% | 93.33 | 6.405 | 8.252 |
| 0.2% | 86.67 | 4.680 | 6.278 |
| 0.3% | 75.00 | 3.615 | 5.345 |
| 0.4% | 63.33 | 2.335 | 3.737 |
| SE | ± 1.63 | ± 0.101 | ± 0.103 |

**Germination:** ILC 482 exhibited the highest overall seed germination of 97.2 as compared to 86.5 and 86.0% of ILC 3279 and ILC 6104, respectively (Table 1). Germination was not affected in ILC 482 and ILC 6104 upto 60 kR and from 70-110 kR germination decreased with increasing doses. Al-Rubeai and Godward (1982), when treated dry seeds of four French bean (*Phaseolus vulgaris* L.) genotype with six different doses of gamma irradiation, from 2.5 to 30 kR, germination was not much affected by even the highest doses. In ILC 3279 germination decreased gradually with increasing doses from 10-110 kR. Reduced germination with increasing doses of gamma irradiation have been reported in chickpea *Cicer arietinum* L. (Shaikh *et al.*, 1980), lentil *Lens culinaris* Medic. (Shaikh *et al.*, 1980; Sharma and Sharma, 1986), mungbean *Vigna radiata* L.

**Table 2. Analysis of variance for the effect of different doses of gamma irradiation and ethyl methanesulphonate (EMS) on germination shoot and root length in chick-pea genotypes**

**a. Combined overall genotypes**

| Source of variation | d.f.[a] | Mean squares | | |
|---|---|---|---|---|
| | | Germination | Shoot length | Root length |
| Replications | 3 | 13.73 | 1.540 | 3.600 |
| Genotypes | 2 | 2724.02** | 1669.015** | 1125.000** |
| Medium vs large seeded (SL) | 1 | 1529.66** | 3336.706** | 2178.000** |
| Within medium seeded (SS) | 1 | 3918.38** | 0.994 | 72.476** |
| Error (a) | 6 | 22.06 | 1.091 | 1.560 |
| Mutagen | 16 | 1271.75** | 266.954** | 504.300** |
| Gamma vs EMS | 1 | 3304.71** | 0.477 | 50.940** |
| Gamma | 11 | 613.64** | 299.087** | 568.611** |
| Linear | 1 | 6117.48** | 3156.790** | 6076.497** |
| Quadratic | 1 | 468.76** | 26.861** | 0.191 |
| Cubic | 1 | 3.43 | 89.217** | 126.558** |
| Quart | 1 | 22.79 | 11.825** | 29.985** |
| Deviations | 7 | 137.54** | 0.752 | 3.070** |
| EMS | 4 | 2573.33** | 245.208** | 440.691** |
| Linear | 1 | 10083.33** | 973.846** | 1716.365** |
| Quadratic | 1 | 192.86** | 0.035 | 33.600** |
| Cubic | 1 | 0.00 | 2.196 | 8.497** |
| Deviations | 1 | 17.14 | 4.754** | 4.301** |
| Genotype x Mutagen | 32 | 226.62** | 13.550** | 10.780** |
| SL x Mutagen | 16 | 342.68** | 24.939** | 15.870** |
| SS x Mutagen | 16 | 110.57** | 2.162** | 5.687** |
| Genotype x Gamma vs EMS | 2 | 1472.65** | 3.222** | 1.230 |
| SL x Gamma vs EMS | 1 | 2923.68** | 2.721* | 2.035 |
| SS x Gamma vs EMS | 1 | 21.62 | 3.722* | 0.430 |
| Genotype x Gamma | 22 | 109.09** | 13.465** | 12.176** |
| SL x Gamma | 11 | 123.86** | 24.533** | 17.090** |
| SS x Gamma | 11 | 94.32** | 2.397** | 7.258** |
| Genotype x Linear (Gamma) | 2 | 747.38** | 129.940** | 106.792** |
| SL x Linear (Gamma) | 1 | 531.03** | 249.184** | 164.799** |
| SS x Linear (Gamma) | 1 | 963.72** | 10.696** | 48.785** |
| Genotype x Quadratic (Gamma) | 2 | 268.93** | 5.187** | 1.160 |
| SL x Quadratic (Gamma) | 1 | 499.50** | 1.566 | 0.179 |
| SS x Quadratic (Gamma) | 1 | 38.36 | 8.807** | 2.142 |
| Genotype x Cubic (Gamma) | 2 | - | 2.242 | 0.244 |
| SL x Cubic (Gamma) | 1 | - | 2.526* | 0.400 |
| SS x Cubic (Gamma) | 1 | - | 1.957 | 0.088 |
| Genotype x Quadratic (Gamma) | 2 | - | 2.242 | 0.244 |

| | | | | |
|---|---|---|---|---|
| Genotype x Deviation (Gamma) | 14 (18) | 20.41 | 1.343** | 3.217** |
| SL x Deviation (Gamma) | 7 (9) | 36.88 | 2.073** | 2.831** |
| SS x Deviation (Gamma) | 7 (9) | 3.93 | 0.613 | 3.604** |
| Genotype x EMS | 8 | 238.33** | 16.368** | 9.322** |
| SL x EMS | 4 | 299.17** | 31.609** | 15.970** |
| SS x EMS | 4 | 177.50** | 1.126 | 2.678** |
| Genotype x Linear (EMS) | 2 | 615.83** | 63.630** | 31.226** |
| SL x Linear (EMS) | 1 | 570.42** | 123.649** | 62.060** |
| SS x Linear (EMS) | 1 | 661.25** | 3.610* | 0.391 |
| Genotype x Quadratic (EMS) | 2 | 301.79** | 0.082 | 3.301** |
| SL x Quadratic (EMS) | 1 | 602.68** | 0.094 | 0.066 |
| SS x Quadratic (EMS) | 1 | 0.89 | 0.069 | 6.536** |
| Genotype x Cubic (EMS) | 2 | - | 0.770 | 2.613* |
| SL x Cubic (EMS) | 1 | - | 1.534 | 1.710 |
| SL x Cubic (EMS) | 1 | - | 0.006 | 3.156* |
| Genotype x Deviations (EMS) | 2 (4) | 23.75 | 0.990 | 0.148 |
| SL x Deviation (EMS) | 1 (2) | 23.57 | 1.160 | 0.026 |
| SS x Deviation (EMS) | 1 (2) | 23.93 | 0.819 | 0.270 |
| Error (b) | 144 | 33.17 | 0.609 | 0.638 |
| Error (sampling) | 816 | - | 0.209 | 0.273 |
| Total | 1019 (203) | 187.82 | 8.157 | 10.795 |

*, ** Significant at P = 0.05 and 0.01 levels, respectively.
Gamma = Gamma irradiation; a = wherever d.f. is different for three cases, d.f. in parenthesis is for germination; - = not obtained.

**b. Genotype-wise**

| Source of | d.f. | Mean squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | Shoot length | | | Root length | | |
| | | Genotype | | | Genotype | | |
| | | ILC 382 | 8LC 3279 | ILC 6104 | ILC 482 | ILC 3279 | ILC 6104 |
| Replications | 3 | 0.340 | 3.209 | 0.172 | 3.467 | 3.175 | 0.080 |
| Mutagens | 16 | 115.658** | 147.052** | 31.345** | 182.174** | 243.333** | 100.327** |
| Gamma vs EMS | 1 | 0.085 | 0.852 | 0.899 | 26.698** | 17.972** | 8.738** |
| Gamma | 11 | 127.030** | 160.756** | 38.231** | 193.154** | 284.412** | 115.396** |
| Linear | 1 | 1337.398** | 1669.080** | 382.192** | 2052.772** | 3045.411** | 1192.000** |
| Quadratic | 1 | 1.973 | 31.378** | 3.882** | 36.329** | 41.557** | 49.160** |
| Cubic | 1 | 50.290** | 26.142** | 17.268** | 36.329** | 41.557** | 49.160** |
| Quartic | 1 | 5.148** | 2.759 | 4.106** | 25.603** | 0.357 | 14.650** |
| Deviations | 7 | 0.360 | 1.565 | 1.870** | 1.298 | 5.710** | 1.899** |
| EMS | 4 | 113.281** | 144.643** | 20.018** | 190.847** | 186.704** | 81.783** |
| Linear | 1 | 450.000** | 571.220** | 79.885** | 760.500** | 712.531** | 305.786** |
| Quadratic | 1 | 0.002 | 0.175 | 0.020 | 2.057 | 25.501** | 12.644** |
| Cubic | 1 | 2.000 | 1.711 | 0.024 | 0.031 | 6.125* | 7.566** |
| Deviations | 1 | 1.120 | 5.469* | 0.144 | 0.802 | 2.658 | 1.1387* |
| Error | 48 | 0.642 | 1.013 | 0.170 | 0.663 | 1.061 | 0.190 |
| Error (Samp) | 272 | 0.300 | 0.284 | 0.042 | 0.315 | 0.399 | 0.105 |
| Total | 339 | 5.793 | 7.340 | 1.837 | 8.976 | 11.983 | 4.847 |

*, ** Significant at P = 0.05 and 0.01 levels, respectively.

Wilczek, mash *Vigna mungo* L. Hepper, and wild mash *Vigna sublobata* Roxb. (Ignacimuthu and Babu, 1988), pigeonpea *Cajanus cajan* L. Millsp. (Venkateswarlu *et al.*, 1981). On the basis of germination ILC 482 appeared more radioresistant and the large seeded genotype ILC 6104 more radiosensitive.

In case of EMS treatment, ILC 482 exhibited 100% germination at 0.1 and 0.2% while in ILC 3279 and ILC 6104 germination decreased linearly with the increasing EMS doses. Differences in varietal response to different EMS doses have also been reported in Faba bean *Vicia faba* L. When seed of Rebaye 40 and Giza 4 were treated with EMS, increasing dosage did not affect germination in Rebaye 40, but in Giza 4 the effect was directly proportional to dosage (Hussein *et al.,* 1974). In pigeonpea, germination in two genotypes treated with 0.02, 0.04, 0.06 M EMS decreased linearly with increased doses (Venkateswarlu *et al.*, 1981). Germination was significantly ($P<0.01$) affected by gamma irradiation and EMS treatment (Table 2) genotype x mutagen interactions and genotypic differences between medium seeded (ILC 482, ILC 3279) versus large seeded (ILC 6104) genotypes were significant ($P<0.01$). On the basis of germination, the ILC 482 appeared more EMS resistant and ILC 6104 as least EMS resistant. Reduction in germination might be due to an increase in the production of active radicals responsible for seed lethality.

**Shoot length:** ILC 482 exhibited the highest overall shoot length of 6.2 cm as compared to 6.1 and 2.3 cm of ILC 3279 and ILC 6104, respectively (Table 1). At lower doses of gamma irradiation a stimulating effect on shoot growth was observed in all the genotypes. In ILC 6104 the stimulating effect was observed at 10, 20, 30 kR in ILC 482 at 10 and 20 kR and in ILC 3279 at 20 kR dose. Stimulating effects of low doses of ionizing irradiation have been reported in chickpea (Khanna and Maherchandani, 1981), wheat and triticale (Hassan *et al.*, 1982). Sax (1963) attributed stimulating effect of low doses to their effect on the auxin balance or to the increased cell expansion with greater mitotic activity in chickpea seedlings raised after gamma irradiation treatment and observed increased activity at lower gamma irradiation doses. Higher doses resulted in decreased activity. Gamma irradiation causes damage to the tissues by producing $H_2O_2$ and organic peroxy radicals, and peroxidase are the internal mechanism for removal of these radicals. The increase in enzyme activity at lower doses could be a response of the tissue to the increase in peroxidase. At higher doses the whole of cellular metabolism is grossly impaired resulting in lower enzyme activity. The shoot length decreased at higher doses with the increasing doses of gamma irradiation. Reduction in shoot length with increasing doses of gamma irradiation have also been reported in chickpea, mungbean, and lentil (Shaikh *et al.*, 1980), and pigeonpea (Nadarajan *et al.*, 1985). The reduction in the shoot length may be attributed to the damage to the process of cell division and cell elongation which generally result after mutagenic treatment (Walther, 1969). Maximum shoot reduction was observed in ILC 6104 (87%) followed by ILC 3279 (82%) and ILC 482 (68%). Large seeded genotype ILC 6104 was more radiosensitive. Sharma and Sharma (1986) have reported that macrosperma genotypes of lentil were more sensitive to gamma rays than microsperma genotypes.

In case of EMS treatments, no stimulating effects were observed and a linear reduction in shoot length occurred in all the genotypes. The maximum reduction was observed at 0.4% in ILC 6104 (73%) followed by ILC 3279 (70%) and ILC 482 (64%). ILC 482 appeared more EMS resistant and ILC

Table 3. Estimation of gamma irradiation and ethyl methanesulphonate (EMS) doses for reduction (GRD) in shoot and root lengths of chickpea genotypes

| | Genotypes | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ILC 482 | | | | ILC 3279 | | | | ILC 6104 | | | |
| A | Est | ESE | Lower | Upper | Est | ESE | Lower | Upper | Est | ESE | Lower | Upper |
| a. Gamma irradiation - Shoot | | | | | | | | | | | | |
| 0.20 | 46.8 | 1.24 | 44.3 | 49.2 | 45.7 | 1.00 | 43.7 | 47.7 | 51.9 | 3.13 | 45.7 | 58.0 |
| 0.30 | 55.7 | 1.08 | 53.7 | 57.9 | 55.2 | 0.86 | 53.5 | 56.9 | 57.9 | 2.87 | 52.2 | 63.5 |
| 0.40 | 64.7 | 1.08 | 62.6 | 66.8 | 64.1 | 0.81 | 62.5 | 65.7 | 63.8 | 2.83 | 58.2 | 69.3 |
| 0.50 | 74.3 | 1.27 | 71.8 | 76.8 | 73.0 | 0.85 | 71.3 | 74.6 | 69.8 | 3.03 | 63.9 | 75.8 |
| b. Gamma irradiation - Root | | | | | | | | | | | | |
| 0.20 | 40.6 | 2.53 | 35.6 | 45.5 | 37.3 | 2.49 | 32.4 | 42.1 | 45.6 | 2.52 | 40.7 | 50.6 |
| 0.30 | 50.4 | 2.15 | 46.2 | 54.6 | 47.5 | 2.06 | 43.4 | 51.5 | 52.7 | 2.21 | 48.3 | 57.0 |
| 0.40 | 60.1 | 2.02 | 56.1 | 64.0 | 57.1 | 1.83 | 53.5 | 60.7 | 59.6 | 2.10 | 55.5 | 63.7 |
| 0.50 | 70.1 | 2.24 | 65.8 | 74.5 | 66.7 | 1.89 | 63.0 | 70.4 | 66.7 | 2.22 | 62.3 | 71.1 |
| c. EMS - Shoot | | | | | | | | | | | | |
| 0.20 | 0.13 | 0.000 | 0.00 | 0.00 | 0.12 | 0.000 | 0.00 | 0.00 | 0.1 | 0.000 | 0.00 | 0.00 |
| 0.30 | 0.19 | 0.003 | 0.18 | 0.19 | 0.18 | 0.004 | 0.17 | 0.19 | 0.16 | 0.001 | 0.16 | 0.16 |
| 0.40 | 0.25 | 0.006 | 0.24 | 0.26 | 0.24 | 0.008 | 0.22 | 0.25 | 0.22 | 0.003 | 0.21 | 0.22 |
| 0.50 | 0.31 | 0.009 | 0.29 | 0.33 | 0.29 | 0.011 | 0.27 | 0.31 | 0.27 | 0.004 | 0.26 | 0.28 |
| c. EMS - Root | | | | | | | | | | | | |
| 0.20 | 0.11 | 0.000 | 0.00 | 0.00 | 0.09 | 0.000 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 |
| 0.30 | 0.17 | 0.002 | 0.17 | 0.18 | 0.16 | 0.006 | 0.15 | 0.17 | 0.14 | 0.005 | 0.13 | 0.15 |
| 0.40 | 0.23 | 0.000 | 0.23 | 0.24 | 0.23 | 0.014 | 0.20 | 0.26 | 0.20 | 0.014 | 0.18 | 0.23 |
| 0.50 | 0.29 | 0.006 | 0.28 | 0.30 | 0.30 | 0.022 | 0.26 | 0.34 | 0.27 | 0.022 | 0.22 | 0.31 |

Est = estimates, ESE = estimated asymptotic standard error, lower and upper are lower and upper 95% confidence limits of GRD.

6104 as the least EMS resistant. The GRD 50 values for shoot length estimated by the model (Table 3) for ILC 482, ILC 3279, and ILC 6104 were 74.3, 73.0, 69.8 kR gamma irradiation and 0.31, 0.29, 0.27% EMS, respectively. Analysis of variance for the effect of different doses of gamma irradiation and EMS on shoot length (Table 2) revealed that genotypic differences were significant ($P<0.01$) between medium seeded versus large seeded genotypes. Genotype x mutagen interactions were also significant ($P<0.01$), indicating that differences exist within medium seeded and between medium and large seeded genotypes regarding sensitivity to mutagenic treatments.

**Root length:** ILC 3279 exhibited the highest overall root length of 7.9 cm compared to 7.2 and 4.5 cm of ILC 482 and ILC 6104, respectively. In this parameter, a trend similar to shoot length was observed. In ILC 6104 the stimulating effect was observed at 10, 20, and 30 kR and in ILC 482 and ILC 3279 at 10 and 20 kR doses. Stimulation effect on root length at lower doses has been reported in mungbean (Song *et al.,* 1988). The root length decreased with increasing doses and maximum reduction in root length was observed in ILC 6104 (82%) followed by ILC 3279 (79%) and ILC 482 (75%). The reduction in root length with increasing doses of gamma irradiation has been reported in wheat and triticale (Hassan *et al.,* 1982).

In case of EMS treatments no stimulating effects were observed and a linear reduction in shoot length occurred in all the genotypes. Maximum root length reduction at 0.4% was observed in ILC 6104 (69%) followed by ILC 482 (66%) and ILC 3279 (62%). The reduction in root length may be the result of marked suppression of mitotic division (Miura *et al.,* 1974). Analysis of variance for the effect of different doses of gamma irradiation and EMS on root length (Table 2) revealed that genotypic differences for root length between medium seeded versus large seeded genotypes and within medium seeded genotypes were significant ($P<0.01$). Genotype x mutagen interactions were also significant ($P<0.01$) indicating that differences exist within and between medium verse large seeded genotypes regarding sensitivity of different mutagenic treatments.

The GRD 50 values for root length estimated by the model (Table 3) for ILC 482, ILC 3279 and ILC 6104 were 70.1, 66.7 and 66.7 kR gamma irradiation and 0.29, 0.30, 0.27% EMS, respectively. The higher doses of gamma irradiation and EMS showed an overall reduction in all the parameters studied. This may be partly due to the fact that the cells which have relatively more chromosomal damage at high irradiation exposures are at a disadvantage due to diplontic selection and can not compete well with the normal cells and are thus prevented from making any further contribution.

From the results, it appeared that out of three parameters studied, shoot and root length can be used with equal reliability for estimating the appropriate doses of gamma irradiation and EMS for treatment on a large scale in a breeding programme. Whatever the reason for a differential behaviour of physical damage may be, for breeding purpose mutagenic treatments with low physiological effects and strong genetic effects are desirable. The physiological damage sets a practical limit to increasing the dose and an end point is reached with 100% lethality. It is for that reason that mutagens are required that result in low plant injury but in high genetic effects. From the GRD doses, it appeared appropriate to use a dose range of 40-60 kR gamma irradiation and 0.1-0.2% EMS which were effective to reduce about 20-40% shoot and/or root length.

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