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RADIO SENSITIVITY OF VARIOUS CHICKPEA GENOTYPES IN M_1 GENERATION I-LABORATORY STUDIES

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Abstract

The effect of gamma rays and EMS mutagens on germination and seedling growth of four chickpea (*Cicer arietinum* L.) genotypes i.e., two desi (Pb2000 and C44), one kabuli (Pb-1) and one desi x kabuli introgression genotype (CH40/91) were investigated. The treatments included two doses each of gamma irradiation and EMS. At lower doses of gamma rays, shoot and root lengths were increased in all four genotypes, whereas adverse effects were observed at higher doses/concentrations of gamma irradiation and EMS. The sensitivity of gamma irradiation and EMS appeared to be related to the seed size and type of genotypes. The desi x kabuli introgression (CH40/91) genotype was the most sensitive to physical mutagen than bold seeded desi and small seeded kabuli genotypes. The trend of radio and chemo resistance was in the order i.e., C44>Pb2000>Pb-1>CH40/91. The present study of radiosensitivity and response of desi x kabuli chickpea introgression genotype to the physical and chemical mutagens is the first report in chickpea.

Introduction

Chickpea acquires importance for cheap vegetable protein, good source of carbohydrates, low cost of production and capacity for fixing atmospheric nitrogen. It plays pivotal role of supplying protein source in the vegetarian diet and is also called "poor man's meat". Supplementation of cereals with high protein legume is potentially one of the best solutions to protein calorie malnutrition, particularly in Pakistan and other developing countries.

With the discovery of ionizing and chemical mutagens a new field of science known as "mutation breeding" was developed. These mutagens may cause genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants. Induced mutations provide beneficial variations for practical plant breeding purpose. During the past seven decades, more than 2252 mutant varieties have been officially released in the world (Maluszynski, *et al*., 2000). A great majority of mutant varieties (64%) were developed by the use of gamma rays (Ahloowalia *et al.*, 2004). Among the chemical mutagen, EMS is reported to be the most effective and powerful mutagen (Minocha & Arnason, 1962; Hajra, 1979). In plants, EMS usually causes point mutations (Okagaki *et al.*, 1991). Khatri *et al.*, (2005) reported that gamma rays and EMS could be fruitfully applied to develop new varieties with high yield and other improved agronomic traits.

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 that may lead to death of the organism. A relatively lower dose often results in altered growth characteristics (Davis, 1970; Fowler & Mac Queen, 1972). With respect to radiation doses the terms lower and higher is 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 (Kwon & Im, 1973). Seed germination, seedling growth and chromosomal aberration are the commonly used criteria for radiosensitivity in plants and in certain cases M_1 sterility has been shown to be appropriate criterion (Kivi, 1964).

The present study was carried out to obtain practical knowledge about the effectiveness of gamma irradiation (physical mutagen) and EMS (chemical mutagen) in M_1 generation in 4 chickpea genotypes (Pb2000, C44, Pb-1 and desi x kabuli introgression line CH40/91) and to estimate the mutagen doses effective to reduce the growth by a given proportion under control conditions.

Materials and Methods

Laboratory experiment

Seed material: The seed material used for this study comprised of 3 commercial and one advance chickpea genotypes viz., two desi (Pb2000 and C44), one kabuli (Pb-1) and one recombinant line of desi x kabuli introgression (CH41/91 or P40/91).

Healthy dry seeds of 4 genotypes were taken from the same harvest and graded to a uniform size at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan during October 2000. Seeds were given EMS treatment in the laboratory and gamma irradiation treatment in the gamma cell Lab. Moisture content of the seeds at the time of mutagenic treatments was maintained at 10 to 12% by keeping them in a desiccator containing Calcium chloride.

Gamma irradiation treatment: Prior to the mutagenic treatments, all genotypes were grown for one generation to ensure their homozygosity. The well developed seeds of same age were selected and subjected to gamma irradiation doses of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 and 1200 Gy (Gray) (40 seeds for each treatment in four genotypes). Gamma irradiation was carried out at NIAB at room temperature (22-25^{o}C) in a Cobalt^{60} gamma cell-220 (Atomic Energy of Canada Ltd.) of 381.43 curie strength delivering 29kR/hr at the time of irradiation.

Ethyl methanesulphonate (EMS) treatment: Seeds were soaked in distilled water in glass beaker for two hours under continuous aeration, before the chemical treatment. The seeds were removed from the beaker and adsorbed water was removed by pressing under folds of blotting paper. The soaked seeds were immediately transferred to freshly prepared 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8% aqueous solution of EMS in distilled water for two hours with regular shaking and aeration at 25 ± 2^{o}C as described by Hussein (1968). The enough volume of mutagenic solution was used in each treatment so that the seeds were completely immersed. The control (non-treated) seeds were soaked in distilled water and were given the same environment as the chemical treatment. The treated seeds were removed from the solution after two hour and were thoroughly washed in running tap water for an hour to leach out the residual chemical and then were dried on blotting paper.

Lay out of experiment: The treated and control seeds were sown on the same day in plastic sigma pots containing sterilized sand in laboratory conditions at Mutation Breeding Division, NIAB, Faisalabad. The experiment was laid out in a completely randomized design with three replications in order to determine the mutagenic effects on seedlings (Sigurbrjőnsson, 1983). Ten seeds per replication per treatment of each

genotype were used. The sigma plant cell culture pots (11 cm long and 11.5 cm in diameter) were placed in an incubator maintained at 25 ± 1ºC.

Germination and seedling growth: The observations on germination were taken on 6th day after sowing by visually counting the number of germinated seeds for each treatment. Shoot and primary root length of seedlings were recorded for 5 randomly selected seedlings from each treatment.

The data was subjected to analysis of variance and growth reduction doses for shoot and root length were determined by using computer software MSTAT-C.

Results and Discussion

Laboratory experiment: Mean germination percentage, shoot length and root length in 4 chickpea genotypes after treatment with different doses of gamma irradiation and EMS are presented in Table 1.

Germination percentage: C44 exhibited the highest overall seed germination percentage of 93.1 as compared to 89.4, 81.4 and 76.2% of Pb2000, Pb-1 and CH40/91, respectively, (Table 1b). Germination was not affected in Pb2000 at gamma radiation doses upto 300 Gy, C44 upto 600 Gy, Pb-1 and CH40/91 upto 200Gy (Table 1a & 1B). Germination showed highly significant negative correlation (-0.988) with gamma rays (Table 4). The result was obtained by plotting the reduction percentage of germination due to radiation/EMS [germination (%) vs radiation dose Gy/EMS (%)] (Figs. 1&2). It is clear that all the four genotypes showed negligible effect of lower doses of gamma irradiation. The desi genotype C44 gave maximum and desi x kabuli introgression line CH40/91 showed minimum effect on the germination percentage. These results are similar to Haq *et al.*, (1992) who reported negligible effect on the germination percentage at the lower doses of gamma irradiation in three kabuli chickpea genotypes (ILC482, ILC3279 and ILC6104). The germination percentage decreased gradually with increasing doses from 400-1200 Gy in Pb2000, from 700-1200 Gy in C44, from 300-1200Gy in Pb-1 and CH40/91. Kumar & Mishra (2004) reported that in okra (*Abelmoschus esculentus*) germination percentage generally decreased with increasing gamma ray dose. Kumar *et al.,* (2003) determined the effects of gamma radiation (200, 400, 600, 800 and 1000 Gy) and ethyl methanesulfonate (10, 20, 30, 40 and 50 mM EMS) on the germination, growth and survival of *Phaseolus lunatus*. They found that the germination and survival % decreased with increasing rates of gamma radiation and EMS. Cheema & Atta (2003) used three basmati rice cultivars to examine varietal differences in radiosensitivity to gamma radiation. They found that with the increase in radiation dose, a decrease in germination under field conditions was observed in M_1 generation although the gamma ray doses had some stimulatory effects on total spikelets at the maturity stage. Reduced germination percentage with increasing doses of gamma irradiation have been reported in chickpea (Haq *et al.,* 1992; Toker & Cagirgan, 2004; Khan & Wani, 2005; Toker *et al.,* 2005), okra (Dhankhar & Dhankhar, 2004), lentil (Kumar & Sinha, 2003), *Solanum trilobatum* (Madhavan & Balu, 1999), limabean (Kumar *et al.,* 2003), cumin (Koli *et al.,* 2002), rice (Pons *et al.,* 2001), sweet potato (Tabares & Talavera, 2003), cowpea (Uma & Salimath, 2001; Gaur *et al.,* 2003) and rye (Akgün & Tosun, 2004). Reduced growth has been attributed to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances (Gunckel & Sparrow, 1954; Gordon, 1957; Singh, 1974; Usuf & Nair, 1974).

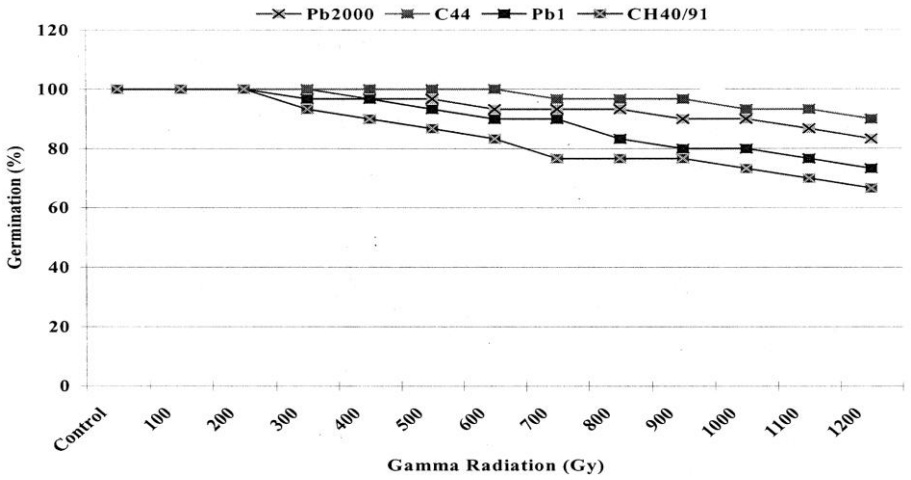

Fig. 1. Dose response relationship for germination percentage in four chickpea genotypes after treatment with different doses of gamma irradiation

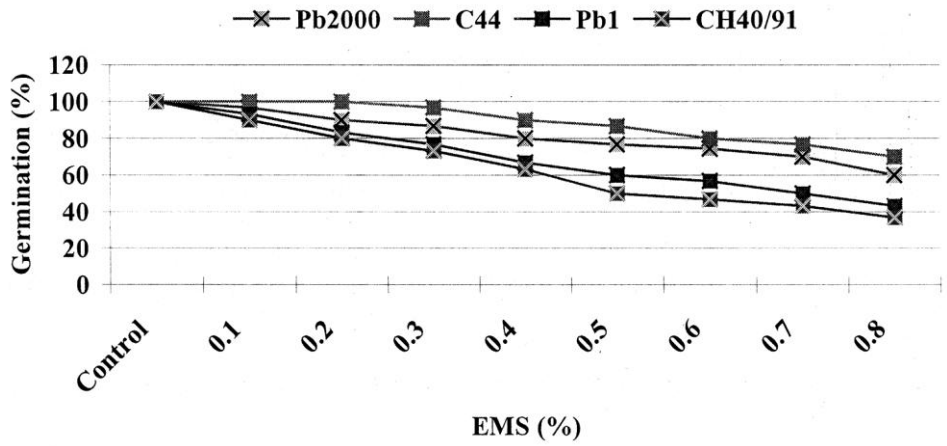

Fig. 2. Dose response relationship for germination percentage in four chickpea genotypes after treatment with different doses of Ethylmethanesulphonate (EMS)

The bold seeded desi genotype C44 appeared more radioresistant while the desi x kabuli introgression genotype CH40/91 appeared more radiosensitive. The CH40/91 used in this study was bold seeded genotype and Haq *et al.,* (1992) and Toker & Cagirgan (2004) also used bold seeded genotypes in their studies. But in the present study bold seeded line was the recombinant of desi x kabuli introgression (CH40/91) while Haq *et al.,* (1992) and Toker & Cagirgan, (2004) used the bold seeded kabuli genotypes. It appeared that bold seeded (CH40/91) genotype was far more sensitive than small seeded kabuli genotype (Pb-1) and bold seeded desi genotypes C44 and Pb2000, to the physical mutagen. Toker *et al*., (2005) reported that the kabuli genotype was more affected to physical mutagen than desi genotype. Our results revealed that the desi x kabuli introgression (CH40/91) genotype was more affected than kabuli and desi genotypes to

physical mutagen. The trend of of radio resistance on the germination percentage in four genotypes was in the order i.e., C44>Pb2000>Pb-1>CH40/91. The study of radiosensitivity and response of germination percentage to physical and chemical mutagen on the recombinant of desi x kabuli chickpea introgression genotype is the first report in chickpea. Our results contradicts the findings of Kumar & Sinha (2003) who observed the small-seeded genotype (Pat L 96-7) in lentil was to be more sensitive to physical mutagen than the large-seeded genotype Precoz.

In case of chemical mutagen, C44 exhibited 100% germination at 0.1 and 0.2% EMS treatments while Pb2000, Pb-1 and CH40/91 displayed linear decrease in percent germination with the increase in EMS doses (Table 1a & 1B). Germination showed highly significant negative correlation (-0.996) with EMS (Table 4). Differences in varietal response to different EMS doses have also been reported in mung bean cultivars (PS-10 and PS-16) by Wani and Khan (2004) where seed germination linearly decreased with increasing concentration of EMS. The maximum inhibition of seed germination in PS-10 and PS-16 (18.75 and 25.26%, respectively) was observed at 0.04% EMS. In two soyabean cultivars, treated with 0.2 and 0.4% EMS, the germination percentage decreased linearly with increasing doses in the M_1 and M_2 generation (Dhole, 2003). Sharma & Kumar (2004) observed that all cultivars showed general reduction in seed germination and survival with increasing concentrations of EMS. The meiotic abnormalities increased along with the increase in concentration of EMS in both cultivars. However, KPG-59 showed more chromosomal abnormalities compared with CSG-89.62. A linear relationship between increasing doses of EMS and reduction in biological trait, such as germination have been reported in chickpea (Toker *et al.,* 2005; Kharkwal, 1999; Haq *et al.,* 1992), red gram (Potdukhe, 2004), okra (Singh *et al.,* 2000), rice bean (Prakash & Shambhulingappa, 2000), mulberry (Hanumantharaju *et al.,* 2000), durum wheat (Kalia *et al.,* 2001), mung bean and urdbean (Rakshit *et al.,* 2001), *Crambe abyssinica* (YouPing *et al.,* 1998), *Nigella sativa* L., (Mitra & Bhowmik, 1998), *Brassica juncea* L (Kumar & Haidar, 1998) and lentil (Waghmare & Mehra, 1998).

Analysis of variance for the effect of different doses of gamma irradiation and EMS on germination is given in Table 3a. In the four genotypes germination percentage was highly significantly ($p<0.01$) affected by gamma irradiation and EMS treatments. Mutagen x genotype, genotype x radiation and genotype x EMS interactions were significant ($p<0.01$). Genotype wise analysis also revealed the highly significant differences between different mutagenic treatments in each genotype (Table 3b). On the basis of germination percentage, the desi type genotype C44 appeared more EMS resistant and desi x kabuli introgression line CH40/91 as least EMS resistant. Reduction in germination percentage might be due to an increase in the production of active radicals responsible for seed lethality.

Shoot length: Seedling shoot length is widely used as an index in determining the biological effects of various physical and chemical mutagens in M_1 (Konzak *et al.,* 1972). The present study showed that C44 exhibited the highest overall shoot length of 7.9 cm as compared to 6.0, 5.6 and 5.3 cm of Pb-1, Pb2000 and CH40/91, respectively (Table 1b). A stimulating effect on shoot growth was observed at lower doses of gamma irradiation in Pb2000 and C44 genotypes. The results were obtained by plotting the reduction percentage of shoots and roots length against radiation dose on graph paper [Length (cm) vs radiation dose (Gy)] (Figs. 3 & 4). In C44 and Pb2000 the stimulating effect was observed at 100 Gy while in Pb-1 and CH40/91 this effect was not seen. Stimulating effects of low doses of ionizing radiation have been reported in chickpea (Haq *et al.,* 1992; Khan & Wani 2005), winged bean (Bai & Sunil 1993), *Bambusa arundinacea*

(Lokesha *et al.*, 1994), soybean (De la *et al.*, 2000) and rice (Cheema & Atta, 2003; Wang *et al.*, 1993). Cepero *et al.*, (2001) observed maximum stimulating effect of low doses (150 and 180 Gy) on seedling height of *Leucaena leucocephala* cv. Cunningham. The lower doses of gamma radiation are concluded to produce a stimulus on the growth of the aerial as well as the underground parts. Khanna & Maharchandani (1981) measured peroxidases activity in chickpea seedlings raised after gamma irradiation treatment and observed increased activity at lower gamma irradiation doses. Higher doses resulted in decreased activity. They reported that gamma radiation apparently causes damage to the tissues by producing H_2O_2 and organic peroxy radicals and peroxidase is 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 peroxides. At higher doses whole of the cellular metabolism is grossly impaired resulting in lower enzyme activity.

Table 1(b). Overall mean germination, shoot length and root length in four chickpea genotypes after treatment with different doses of gamma irradiation and Ethyl methane sulphonate (EMS) in laboratory experiment.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)
Genotype	**One way table of means**		
Pb2000	89.4	5.6	7.2
C44	93.1	7.9	10.3
Pb1	81.4	6.0	9.2
CH40/91	76.2	5.3	8.1
SE	**±0.6517**	**±0.0335**	**±0.033**
Radiation (Gy)			
Control	100	10.3	14.3
100	100	9.9	13.8
200	100	9.1	12.7
300	97.5	8.1	11.1
400	95.8	7.3	10.1
500	94.2	6.6	9.1
600	91.7	5.8	7.9
700	89.2	5.1	6.9
800	87.5	4.6	5.9
900	85.8	3.7	4.9
1000	84.2	3.1	4.1
1100	81.7	2.5	3.4
1200	78.3	1.8	2.7
(EMS%)			
Control	100	10.2	14.3
0.1	95.0	9.5	13.0
0.2	88.3	8.5	11.8
0.3	83.3	7.7	10.7
0.4	75.0	6.6	9.4
0.5	68.3	5.6	8.0
0.6	66.7	4.6	6.8
0.7	60.0	3.4	5.3
0.8	52.5	2.5	4.1
SE	**± 1.5284**	**± 0.0786**	**± 0.0789**

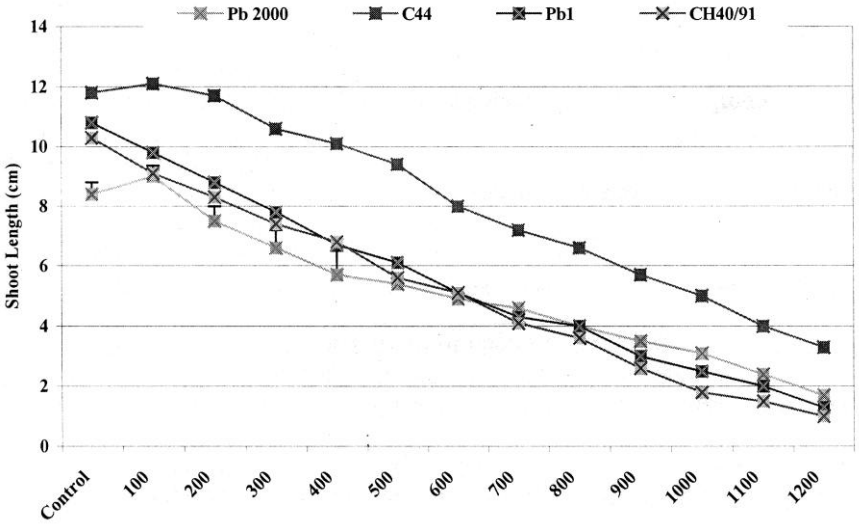

Fig. 3. Dose response relationship for shoot length in four chickpea genotypes after treatment with different doses of gamma irradiation

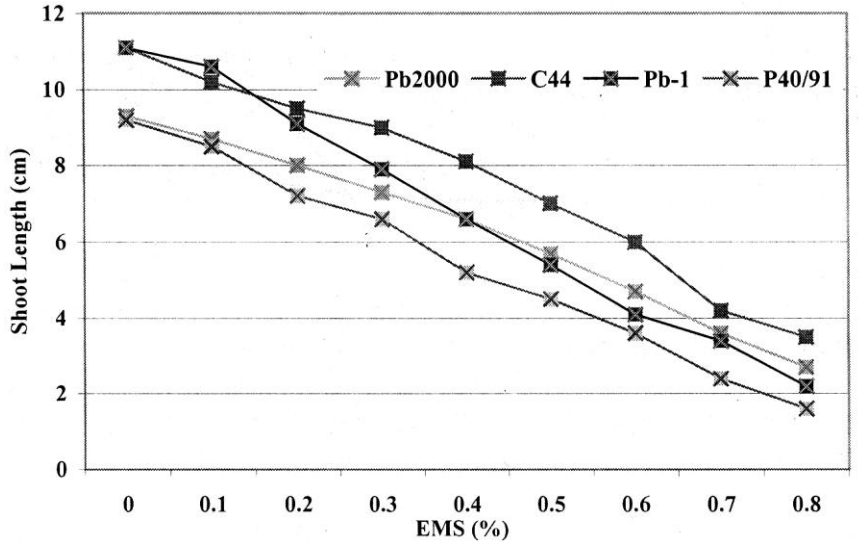

Fig. 4. Dose response relationship for shoot length in four chickpea genotypes after treatment with different doses of Ethyl methanesulphonate (EMS).

Highly significant differences among four genotypes in response to radiation doses were observed for shoot length. The shoot length decreased at higher doses with the increasing doses of gamma irradiation. Shoot length showed highly significant negative correlation (-0.998) with gamma rays (Table 4). Reduction in shoot length at higher doses of gamma irradiation have also been reported in perennial rye seeds (Akgün & Tosun, 2004), basmati rice (Cheema & Atta, 2003), rice (Pons *et al*., 2001), mungbean and urdbean

(Rakshit *et. al*, 2001), *Brassica juncea L.* (Kumar & Haidar, 1998), *Leucaena leucocephala* (Cepero *et al.,* 2001), *Lathyrus sativus* L. (Kumar & Dubey, 1998), barley (Arumugam *et al.,* 1997) and greengram (Kumar & Mishra, 1999). The reduction in the shoot length may be attributed to the damage to the process of cell division and cell elongation that generally result after mutagenic treatment (Iqbal, 1969; Walther, 1969). The trend of maximum shoot reduction in four genotypes was observed in CH40/91 (90.3%) followed by Pb-1 (87.4%), Pb2000 (79.8%) and C44 (72.0%). [CH40/91> Pb-1>Pb2000>C44]. The bold seeded desi x kabuli introgression line CH40/91 was more radiosensitive. Kumar & Sinha (2003) reported in lentil that large-seeded genotype Precoz was more sensitive to mutagenic treatments in contrast to small-seeded genotype Pat L 96-7.

After EMS treatments the maximum overall reduction in shoot length expressed as percent of control was observed in CH40/91 (82.6%) followed by Pb-1 (80.2%), Pb2000 (70.9%) and C44 (68.5%). C44 appeared more EMS resistant and CH40/91 as least EMS resistant (Table 1a & 1b). GRD 50 values for shoot length estimated by the model (Table 2 a & b) for C44, Pb2000, Pb-1 and CH40/91 were 884, 770, 624, 624 Gy gamma irradiation and 0.62, 0.59, 0.50, 0.49% EMS, respectively. The graphic representation of dose response relationships for shoot length is shown in Figs. 3 and 4. In case of EMS treatments no stimulating effects were observed and a linear reduction in shoot length occurred in all the genotypes. Shoot length showed highly significant negative correlation (-0.998) with EMS (Table 4). Reduction in shoot length with increasing doses of EMS has been reported in chickpea (Haq *et al.,* 1992; Toker *et al.,* 2005), durum and bread wheat (Kalia *et. al*., 2001). Rakshit & Singh, (2001) used three cultivars each of mung bean (*Vigna radiata)* and urdbean (*V. mungo*) for mutation studies using five doses of EMS. A linear relationship between increasing doses of EMS and reduction in different biological traits such as germination, seedling height, survival at maturity and pollen fertility was recorded at all treatments of EMS.

Analysis of variance for the effect of different doses of gamma irradiation and EMS on shoot length revealed highly significant mutagens and genotypic differences (Table 3a &b). Mutagen x genotype and genotype x radiation/EMS interactions were also significant ($p<0.01$), indicating that differences exist within desi type and kabuli type genotypes regarding sensitivity to mutagenic treatments.

Root length: C44 exhibited highest overall root length of 10.3 cm as compared to 9.2, 8.1 and 7.2 cm of Pb-1, CH40/91 and Pb2000 respectively (Table 1b). At lower doses of gamma irradiation a stimulating effect on root length was observed in desi genotypes viz., C44 and Pb2000. In C44, the stimulating effect was observed at 100 and 200 and in Pb2000 it was observed on the 100 Gy dose. At higher doses root length decreased with increasing doses and overall maximum root length reduction as percent of control was observed in CH40/91 (88.6%) followed by Pb-1 (85.2%), Pb2000 (81.8%) and C44 (70.4%) (Table 1a). Root length showed highly significant negative correlation (-0.997) with gamma rays (Table 4). Stimulation effect in root length at lower doses has been reported in chickpea (Haq *et al.,* 1992; Toker *et al.,* 2005), onion (Amjad & Anjum, 2002), *Brassica juncea* (Kumar & Haidar, 1998) and rice (Pons *et al.,* 2001). The reduction in root length with increasing doses of gamma irradiation has been reported in onion (Amjad & Anjum, 2002), japonica rice (Wang *et al*., 1993; Majeed, 1997), rice (Ashraf *et al.,* 2003; Cheema & Atta, 2003) and cowpea (Uma & Salimath, 2001).

Table 2(a). Estimation of gamma irradiation (Gy) and Ethyl methanesulphonate (EMS) doses for growth reduction (GRD) in shoot and root length of two desi chickpea genotypes.

GRD%	Genotype							
	Pb 2000				C 44			
	Est	Ese	Lower	Upper	Est	Ese	Lower	Upper
	(a) Gamma irradiation- Shoot							
20	329	23.8	277	381	429	14.9	396	462
30	476	20.4	431	521	581	14.2	549	612
40	623	19.3	581	666	732	14.7	700	765
50	770	21.0	724	816	884	16.1	849	919
	(b) Gamma irradiation-Root							
20	300	19.2	257	342	422	16.0	387	458
30	436	16.7	399	472	570	15.4	536	604
40	572	15.5	53.	606	718	15.7	683	752
50	708	15.9	673	743	865	16.7	828	902
	(c) EMS-Shoot							
20	0.26	0.008	0.24	0.28	0.27	0.012	0.24	0.30
30	0.37	0.007	0.35	0.39	0.38	0.011	0.35	0.41
40	0.48	0.008	0.46	0.50	0.50	0.012	0.47	0.53
50	0.59	0.009	0.57	0.61	0.62	0.013	0.59	0.65
	(d) EMS-Root							
20	0.26	0.005	0.25	0.27	0.27	0.011	0.25	0.30
30	0.38	0.005	0.37	0.39	0.39	0.10	0.37	0.41
40	0.50	0.005	0.49	0.51	0.52	0.011	0.50	0.55
50	0.61	0.006	0.60	0.62	0.64	0.012	0.61	0.67

Est.= Estimates, Ese= Estimated asymptotic standard error, Lower and Upper are lower and upper 95% confidence limits of GRD

In case of EMS treatments no stimulating effects were observed and a linear reduction in root length occurred in all the four genotypes. Maximum root length reduction at 0.8% was observed in CH40/91 (78.3%) followed by Pb-1 (74.1%), Pb-2000 (66.9%) and C44 (64.8%) (Table 1a). Root length showed highly significant negative correlation (-0.988) with EMS (Table 5). The reduction in root length may be the result of marked suppression of mitotic division, affected nuclear condensation causing irregular distribution of chromosomes, bridges and fragmentation (Datta *et al.,* 1992). Waghmare & Mehra (1998) assessed the effects of mutagens in the M_1 generation and reported that EMS treatments produced a higher frequency of chimeras than the gamma ray treatments. YaPing *et al.,* (1996) reported EMS induced chromosomal aberrations of root tip cells and adverse effects on seedling ratio, seedling height, root length, root number and peroxidase activity in *Coix lacryma-jobi*. The reduction in root length with increasing EMS concentration has been reported in chickpea (Haq *et al.,* 1992), red gram (Potdukhe, 2004), *Coix lacryma-jobi* (YaPing *et al.,* 1996) and *Vicia hirsuta* (Kumari, 1994).

Table 2(b). Estimation of gamma irradiation and Ethyl methanesulphonate (EMS) doses for growth reduction (GRD) in shoot and root length of two kabuli chickpea genotypes.

GRD%	Genotype							
	Pb 1				CH 40/91			
	Est	Ese	Lower	Upper	Est	Ese	Lower	Upper
	(a) Gamma irradiation- Shoot							
20	210	18.1	170	249	210	13.7	179	240
30	348	15.2	314	381	348	11.5	322	373
40	486	13.2	457	515	486	10.0	464	508
50	624	12.6	596	651	624	09.5	603	645
	(b) Gamma irradiation-Root							
20	193	18.6	152	233	227	14.0	196	25.8
30	334	16.1	299	370	364	12.2	337	39.0
40	476	14.5	444	508	500	11.0	476	52.5
50	618	14.0	587	649	637	10.8	61.3	66.1
	(c) EMS-Shoot							
20	0.21	0.007	0.20	0.23	0.20	0.006	0.19	0.21
30	0.31	0.006	0.30	0.32	0.30	0.005	0.29	0.31
40	0.40	0.006	0.39	0.41	0.39	0.005	0.38	0.40
50	0.50	0.006	0.49	0.51	0.49	0.005	0.48	0.50
	(d) EMS-Root							
20	0.22	0.004	0.21	0.23	0.19	0.005	0.18	0.20
30	0.32	0.004	0.31	0.33	0.30	0.004	0.29	0.31
40	0.43	0.004	0.42	0.44	0.40	0.004	0.39	0.41
50	0.53	0.004	0.52	0.54	0.51	0.004	0.50	0.52

Est.= Estimates, Ese= Estimated asymptotic standard error, Lower and Upper are lower and upper 95% confidence limits of GRD

Analysis of variance for the effect of different doses of gamma irradiation and EMS on root length revealed significant genotypic differences ($p<0.01$) (Table 3a). Genotype x mutagen interactions was also significant ($p<0.01$) indicating that differences exist within genotypes regarding sensitivity of different mutagenic treatments. GRD 50 values for root length estimated by the model (Table 2a,b) for C44, Pb2000, CH40/91and Pb-1 were 865, 708, 637 and 618 Gy gamma irradiation and 0.64, 0.61, 0.51, 0.53% EMS, respectively. The graphic representation of dose response relationship for root length is shown in Figs. 5 and 6. 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.

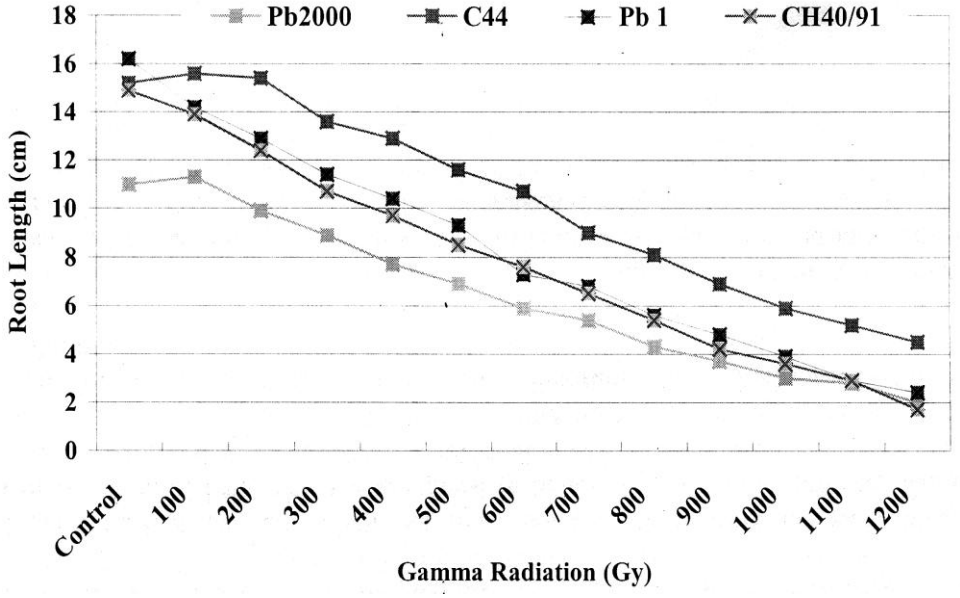

Fig. 5. Dose response relationship for root length in four chickpea genotypes after treatment with different doses of gamma irradiation.

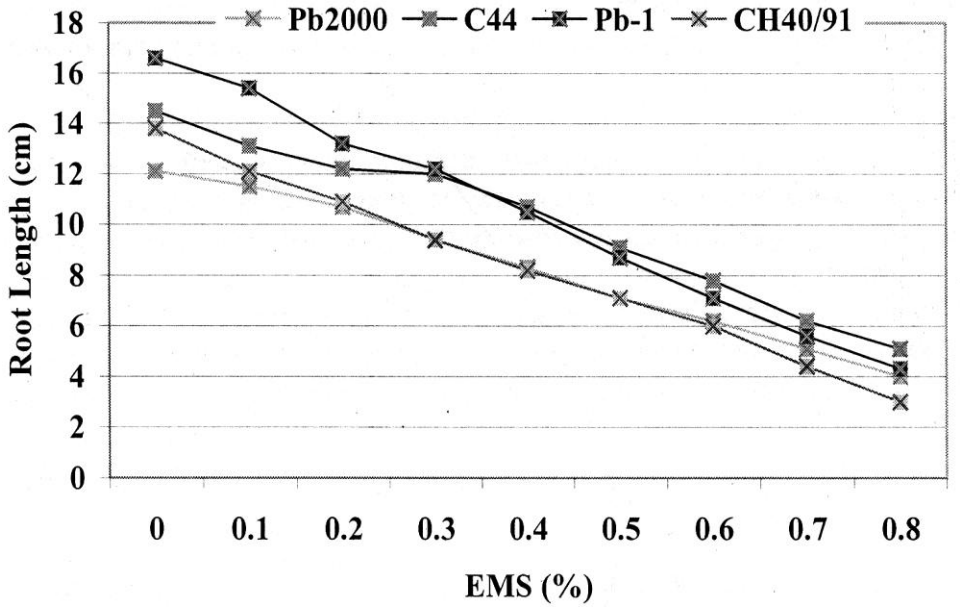

Fig. 6. Dose response relationship for root length in four chickpea genotypes after treatment with different doses of ethyle methanesulphonate (EMS).

Table 4. Correlation of varieties with radiation and EMS doses and their response to mutagens under laboratory conditions.

Plant character	Mutagen	Correlation	Control	Reduction (%)
Germination (%)	Gamma rays	-0.988**	100.00	21.7
	EMS	-0.996**	100.00	47.5
Shoot length (cm)	Gamma rays	-0.998**	10.33	82.2
	EMS	-0.998**	10.18	75.4
Root length (cm)	Gamma rays	-0.997**	14.33	81.5
	EMS	-0.997**	14.25	71.2

From the results it can be inferred that out of three parameters studied, shoot and root length can be used with equal reliability for estimating the suitable doses of gamma irradiation and EMS for treatment on a large scale in a breeding program. The ultimate aim of a mutagenic treatment is to induce mutations leading to genetic improvement of a specific trait and selection of economically important mutants. For breeding purposes mutagenic treatments with low physiological effects and strong genetic effects are desirable. The physiological damage sets a practical limit to increase 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 high in genetic effects.

From the GRD doses it appeared appropriate to use a gamma irradiation dose range of 300-400 Gy for Pb2000, 500-600Gy for C44, 200-300Gy for Pb-1 and CH40/91 and 0.3-0.4% EMS for Pb-2000 and C44 and 0.2-0.3% for Pb-1 and CH40/91 which were effective to reduce 20-40% shoot and or root length. The lower doses were selected as the conditions for plant growth in the laboratory are usually mild but the conditions are relatively harsh under field conditions.

Conclusion

On the basis of two mutagens (gamma rays and EMS) and three parameters studied (germination, shoot and root length), bold seeded desi genotype C44 appeared more radio and chemo resistant while the desi x kabuli introgression genotype CH40/91 appeared more radio and chemo sensitive. The trend of radio and chemo resistance on basis of three parameters in four genotypes was in the order i.e., C44>Pb2000>Pb-1>CH40/91 in the present study. The study of radiosensitivity and response to gamma radiation and EMS of the recombinant of desi x kabuli chickpea introgression genotype is the first report in chickpea.

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