Pak. J. Bot., 40(2): 605-613, 2008.

INDUCED GENETIC VARIABILITY IN CHICKPEA (*CICER ARIETINUM* L.) II. COMPARATIVE MUTAGENIC EFFECTIVENESS AND EFFICIENCY OF PHYSICAL AND CHEMICAL MUTAGENS

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Abstract

Comparative mutagenic effectiveness and efficiency of gamma rays and Ethyl methane sulphonate (EMS) were studied in two desi (Pb2000 and C44), one kabuli (Pb1) and one desi x kabuli introgression line (CH40/91) of chickpea. The treatments included two doses each of gamma rays and EMS calculated on the basis of their LD_{30}. The results revealed that EMS was almost seven times more effective and its efficiency was two times higher than that of gamma rays. Mutagenic effectiveness and efficiency were found to depend upon mutagen type and the genotype and both were higher at lower doses of EMS in three genotypes except in desi genotype C44. The overall trend of mutagenic effectiveness and efficiency in both gamma radiation and EMS was in the order i.e. CH40/91>Pb2000>Pb1>C44. The introgression line desi x kabuli genotype was found to be most resistant towards mutagenic treatments than desi and kabuli types.

Introduction

Chickpea (*Cicer arietinum* L.) is the third important food legume of the world grown in 40 countries over an area of about 11.2 million hectares, with production of 9.2 million tons and average yield of 818 kg/ha (Anon., 2006). The crop is self-pollinated diploid (2n=2x=16) with a comparatively small genome (Arumuganathan & Earle, 1991). Currently the productivity of chickpea is very low and has stagnant in recent years (Anon., 2005). Despite its high morphological variability, genetic variation is limited probably due to its monophyletic descendence from *Cicer reticulatum* (Ladizinsky & Adler, 1976; Lev-Yadun *et al.,* 2000; Abbo *et al.,* 2003).

Although extensive studies have been undertaken on mutagenesis in cereal crops (Rajendra & Mani, 1997; Konzak *et al.,* 1965; Nilan *et al.,* 1965), its utilization was limited for improving pulse crops (Haq *et al*., 2001, 2002; Kharkwal, 1998; Kharkwal *et al.,* 1988; Nadarajan *et al.* 1982). The ethylated agents, ethyl methane sulphonate (EMS) have been found more effective and efficient than physical mutagens in crops like lentil (Gaikwad & Kothekar, 2004), cowpea (John, 1999), *Lathyrus sativus* (Waghmare & Mehra, 2001) and chickpea (Kharkwal, 1998).

A highly effective mutagen may not necessarily show high efficiency and *vice versa*. The higher efficiency of a mutagen indicates relatively less biological damage (seedling injury, sterility etc.,) in relation to mutations induced (Kharkwal, 1998; Sarker, 1985). Selection of effective and efficient mutagen is very essential to recover high frequency of desirable mutations in any mutation breeding studies. Hence, previous knowledge of effectiveness and efficiency of most commonly used chemical mutagens in relation to ionization radiations in a number of genotypes is indispensable to classify the range of doses/concentrations of useful mutagens for mutation breeding program. At present no conclusive information on relative effectiveness and efficiency of different physical and chemical mutagens is available for chickpea.

The present investigation was therefore undertaken to elicit the response of different chickpea genotypes to gamma radiation (a physical mutagen) and EMS (a chemical mutagen).

Materials and Methods

Four diverse chickpea genotypes were used in this study. The desi genotype Pb2000 is semi-erect, brown and bold seeded, hundred-grain weight is 27.4 gram, sensitive to *Botrytis* gray mold (Akhtar *et al.,* 2004). The other desi genotype C44 is erect and tall growing, medium and brownish color grain and hundred-grain weight is 23 gram. This variety is susceptible to iron-deficiency chlorosis that causes a lot of damage in low temperature and in irrigated areas of Punjab and NWFP. The kabuli variety Pb1 is semi-erect, small roundish shaped grain and hundred-grain weight is 16 gram. This variety is also sensitive to iron- chlorosis and grain size does not attract consumers and doesn't fulfill the market demand (Akhtar *et al.,* 2003). The introgression line of white x desi (CH40/91) is semi- spreading and its grain is brownish, bold, owls head shaped and hundred-grain weight is 26 gram.

Fresh seed were treated (moisture level 10-12%) with different doses/concentration of gamma rays and ethyl methane sulphonate (EMS) viz., 300-400 Gy gamma rays and 0.3-0.4% of EMS for Pb.2000, 500-600 Gy gamma rays and 0.3-0.4% of EMS for C44, 200-300 Gy gamma rays and 0.2-0.3% of EMS Pb1 and 200-300 Gy gamma rays and 0.2-0.3% of EMS for CH40/91.

Prior to the mutagenic treatments, LD_{30} values for each genotypes were determined for the biological parameters by using the regression equation $Y= a+ bX$ where Y=damage and X= dose. All genotypes were grown for one generation to ensure their homozygosity. Gamma irradiation treatments were applied with $Cobalt^{60}$ gamma cell-220 at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. All the treatments were administered at the room temperature (22-25^{o}C). EMS (Sigma, USA) was used for preparing aqueous solution of chemical mutagens. Treatments with chemical mutagens were given with intermittent shaking and the volume of solution was maintained during the treatment. The chemical solutions were freshly prepared in distilled water and pH 8.5 was adjusted just before the treatment. The seeds soaked in distilled water were treated as control. After the treatments, seeds were thoroughly washed in running tap water for one hour to leach out the residual chemical and were sown immediately in the field. For each treatment, 1500 seeds were treated and grown as M_1 generation during 2000-2001 in row measuring three meter length and a distance of 15 cm was maintained between plants and 30 cm between rows at the fields of NIAB, Faisalabad. The seedling height reduction in different M_1 treatments was studied following Nilan *et al.,* (1965) and Sharma (1990). The plant survival percentage (L) was computed as the percentage of plants surviving till maturity. The biological damage (lethality/injury) was computed as the reduction in plant survival and plant height. At maturity all the surviving fertile plants were individually harvested to raise M_2 population. M_2 progenies were raised in rows of 4m length spaced 30 cm apart during 2001-2002. The respective non-treated control was also planted after every tenth rows for comparison. Different kinds of chlorophyll mutants (xantha, viridis, chlorina and albina) were scored from emergence till the age of four week in M_2 generation by using modified classification of Lamprecht (1960) and Kharkwal (1998). Mutation frequency was worked out as percent of mutated M_2 progenies and plants as calculated by Konzak *et al*., (1965) and Kharkwal (1998). Both mutagenic effectiveness (mutation per unit dose) and efficiency (rate of mutations to damage or lethality/sterility) were determined by using the following formulae (Konzak *et al.,* 1965):

Mutagenic effectiveness = Mf/tc or Mf/ Gy
Mutagenic efficiency = Mf/L or Mf/S where,
Mf = Percentage (%) of families segregating for chlorophyll mutations
t = Period of treatment with chemical mutagen
c = Concentration of chemical mutagen in terms of percentage (%)
Gy = Gray of physical mutagen
L = Percentage (%) of lethality in M_1
S = Percentage (%) of sterility in M_1

The frequencies of different types of chlorophyll mutations were pooled for each mutagen in order to study the specificity of mutagens.

Results and Discussion

Mutagenic effectiveness: Mutagenic sensitivity is known to be influenced by an array of factors, such as type of mutagen and dose, moisture content of seed, treatment conditions, stage of development, ploidy level and genotype of the material. In comparing the effects of physical and chemical mutagens, reduction of germination, reduction of plant height, delay in the emergence of M_1 seedlings and induction of micro and macro mutations in M_2 were taken as the main indices for the overall response.

The mutagenic effectiveness of two mutagens and response of desi, kabuli and desi x kabuli introgression line was varying (Table 1). 0.2% EMS was most effective in desi x kabuli introgression line CH40/91 (13.25) followed by 0.3% EMS in desi genotype Pb2000 (7.70), 0.2% EMS in kabuli Pb1 (7.25) and 0.4% EMS in C44 (6.10). Thus the trend of mutagenic effectiveness was in the order i.e. CH40/91>Pb2000> Pb1>C44. For gamma radiation 200 Gy in desi x kabuli introgression line CH40/91 (0.00715) followed by 200 Gy in Pb1 (0.00645), 400 Gy in Pb2000 (0.002) and 500 Gy in C44 (0.00184) was most effective. Thus the trend of mutagenic efficiency was different from the trend of chemical mutagen and was in the order of CH40/91>Pb1> Pb2000>C44. At lower doses Pb2000, Pb1 and CH40/91 gave higher response to mutagenic effectiveness of chemical mutagen. In general, the effectiveness decreased with the increase in dose or concentration. With increasing doses of EMS/gamma rays, the values obtained for all the biological criteria for M_1 generation were decreased. The reduction in biological criteria (plant height and survival) may be attributed to a drop in the auxin level (Gordon & Webber, 1955), inhibition of auxin synthesis (Skoog, 1935), chromosomal aberrations (Sparrow, 1961) or due to decline of assimilation mechanism (Quastler & Baer, 1950). Similar trend of decreasing effectiveness with increasing dose of gamma rays and EMS has been reported in chickpea (Kharkwal, 1998), lentil (Solanki, 2005) and mungbean (Solanki & Sharma, 1994). Higher mutagenic effectiveness and efficiency was observed in *Lathyrus sativus* L., at lower doses of EMS than in the gamma irradiation treatments by Waghmare & Mehra (2001) and Kumar *et al.,* (2003). Such difference in the effects of mutagens on different material might be due to the seed metabolism and onset of DNA synthesis. Kundi *et al.,* (1997) reported differential sensitivity within crop and even genotype. The sensitivity depends upon its genetic architecture and the mutagens employed (Blixit, 1970) besides, the amount of DNA, its replication time in the initial stages and degree of heterochromatin. These criteria are responsible for differential mutagenic sensitivity in a crop. Keeping in view, it was observed in M_2 population that EMS was more pronounced in inducing chlorophyll mutations than gamma rays (Shah *et al.*, 2006) and among the spectrum, the viridis (less drastic mutation) was more than that of albina (extreme mutation) as categorized by Westergaard (1960).

On the basis of pooled treatment over genotype, mutagenic effectiveness of EMS was as high as 10.25% and in the range of 5.43 to 5.87% whereas for gamma rays highest was 0.0068% and in the range of 0.0011 to 0.0062% (Table 2). On the basis of pooled mutagen over genotypes and treatment, EMS was about seven times more effective (6.85) than gamma rays (0.00382). On the overall basis 0.2% EMS and 200 Gy of gamma rays were more effective compared to all other treatments. It means that low concentrations/doses of both mutagens were more effective and the competence decreased gradually with increasing dose/ concentrations. At lower dose of gamma rays (200 Gy) there was less lethality (52.31%) but at lower dose of chemical mutagen (0.2% EMS) lethality was very high (63.65%). On the basis of pooled mutagenic effectiveness over genotypes, the highest mutagenic effectiveness (5.47) and lethality (72.44%) were observed in CH40/91 whereas lowest was observed in C44 and Pb1 (Table 3). The highest effectiveness and lethality in introgression genotype CH40/91 ultimately gave the pronounced response on the relative frequency of micro and macro mutations (chlorophyll and morphological) spectrum in the mutated families of M_2.

It was observed that as the mutagenic dose increased the effectiveness decreased. Our findings are in close agreement with the results of other workers (Gupta & Yashvir, 1975; Sharma, 1966; Dixit & Dubey, 1986). The present study indicated higher effectiveness of EMS over gamma rays and this was also demonstrated by earlier workers (Ramulu, 1972; Desai & Bhatia, 1975).

Mutagenic efficiency: The degree of efficiency of two mutagens was varying (Table 1). 0.3% EMS showed highest mutagenic efficiency (0.0447) in Pb2000 followed by 0.4% EMS in C44 (0.0430) and 0.0345 in Pb-1 and 0.0311 in CH40/91. In case of gamma radiation, the highest mutagenic effectiveness was observed at 300 Gy for CH40/91 (0.0279) followed by 200 Gy for Pb1 (0.0253), 500 Gy for C44 (0.0171) and 400 Gy for Pb2000 (0.0148). Thus the trend of mutagenic efficiency was in the order of Pb2000>C44>Pb1>CH40/91 for EMS and CH40/91> Pb1>C44> Pb2000 for gamma rays. The gamma rays appeared less efficient than EMS. Efficiency of mutagenic treatments (treatments pooled over varieties) indicated that 0.4% EMS was most efficient than all other doses of EMS and gamma rays (Table 2). Mutagenic efficiency of EMS was about two times higher than that of gamma rays. This is in agreement with various earlier reports on pulse crops (Rapoport, 1966; Sharma, 1966; Sharma *et al*., 2005).

Lower concentrations of EMS was found to be most efficient in three genotypes CH40/91, Pb1 and Pb2000 while lower doses of gamma radiation were effective in only two genotypes, C44 and Pb1.

Table 1. Mutagenic effectiveness and efficiency based on micro mutations (chlorophyll) induced by gamma radiation and EMS in four chickpea genotypes.

Genotype	Dose	Lethality (%)	Mutated families (%)	Effectiveness	Efficiency
Pb.2000	300 Gy	55.25	0.45	0.0015	0.0081
	400 Gy	54.05	0.80	0.002	0.0148
	0.3 % EMS	51.69	2.31	7.70	0.0447
	0.4 % EMS	57.56	1.90	4.75	0.0330
C44	500 Gy	53.86	0.92	0.00184	0.0171
	600 Gy	52.97	0.75	0.00125	0.0142
	0.3 % EMS	55.36	1.24	4.13	0.0219
	0.4 % EMS	56.69	2.44	6.10	0.0430
Pb. 1	200 Gy	50.89	1.29	0.00645	0.0253
	300 Gy	57.97	1.26	0.00420	0.0217
	0.2 % EMS	42.04	1.45	7.25	0.0345
	0.3 % EMS	51.75	0.89	2.97	0.0172
CH40/91	200 Gy	53.72	1.43	0.00715	0.0266
	300 Gy	66.25	1.85	0.00617	0.0279
	0.2 % EMS	85.25	2.65	13.25	0.0311
	0.3 % EMS	84.50	2.59	8.63	0.0307

Table 2. Mutagenic effectiveness and efficiency based on chlorophyll mutations induced by different mutageic doses and mutagens.

Mutagen	Treatment basis[1]					Mutagen basis[2]	
	Dose	Lethality (%)	Mutation frequency (%)	Effectiveness	Efficiency	Effectiveness	Efficiency
Gamma rays (Gy)	200	52.31	1.36	0.0068	0.0260	0.00382	0.01946
	300	59.82	1.87	0.0062	0.0313		
	400	54.05	0.80	0.0019	0.0148		
	500	52.97	0.92	0.0020	0.0171		
	600	55.36	0.75	0.0011	0.0141		
EMS (%)	0.2	63.65	2.05	10.25	0.0322	6.85	0.03201
	0.3	61.16	1.74	5.87	0.0255		
	0.4	48.57	2.17	5.43	0.0380		

[1]Treatment basis (treatment pooled over varieties)

[2]Mutagen basis (mutagens pooled over varieties and treatments)

Table 3. Total (pooled) mutagenic effectiveness and efficiency of gamma radiation and EMS in four chickpea genotypes.

Genotype	Lethality (%)			Mutated families (%)			Effectiveness			Efficiency		
	Gamma rays	EMS	Overall	Gamma rays	EMS	Overall	Gamma rays	EMS	Overall	Gamma rays	EMS	Overall
Pb2000	54.65	54.63	54.64	0.625	2.11	1.37	0.0018	6.23	3.12	0.0115	0.0389	0.0252
C44	53.42	56.03	54.73	0.835	1.84	1.34	0.00155	5.12	2.56	0.0157	0.0325	0.0241
Pb1	54.43	46.90	50.67	1.275	1.17	1.22	0.00533	5.11	2.56	0.024	0.0259	0.0247
CH40/91	59.99	84.88	72.44	1.64	2.62	2.13	0.0067	10.94	5.47	0.0273	0.0309	0.0291
Mean	**55.62**	**60.61**	**58.12**	**1.09**	**1.94**	**1.52**	**0.00382**	**6.85**	**3.43**	**0.0195**	**0.0320**	**0.0258**

In general, the treatments with low concentrations of EMS were more effective and efficient as measured on the basis of lethality and injury than treatments with higher concentrations. The efficiency of a mutagenic agent is of a complex nature, as it does not only depends on the reactivity of the agent with the material and on its applicability to the biological system but also on the degree to which physiological damage, chromosomal aberrations and sterility are induced in addition to mutations. Kaul (1969) and Veleminsky & Gichner (1970) pointed out that the effectiveness and efficiency of nitroso amides were greatly influenced by pH and temperature during treatment.

The present study indicated that the mutagenic effectiveness and efficiency decreased with the increasing dose of mutagens. Higher efficiency at the lower concentration of the mutagen appears mainly due to the fact that injury, lethality and sterility increases with an increase in the mutagen concentration than actual mutations (Kharkwal, 1998; Cheema *et al*., 2003). Efficient mutagens and their treatments are indispensable for the cost-effective use of the mutagen as a tool for the induction of mutations and their direct and indirect utilization in successful breeding program.

Conclusion

Mutagenic effectiveness and efficiency of gamma rays and EMS were studied using four genotypes of chickpea. EMS was almost seven times more effective than gamma rays whereas its efficiency was two times higher than that of gamma rays. Both mutagenic effectiveness and efficiency were higher at lower doses of EMS in Pb.2000, Pb1 and CH40/91. In case of gamma rays at lower doses, effectiveness was higher in three genotypes (Pb.2000, Pb.1 and CH40/91), whereas efficiency was higher in C44 and Pb1 but was lower in Pb2000 and CH40/91 genotypes. On overall basis, the trend of mutagenic effectiveness and efficiency of both gamma radiation and EMS was in the order of CH40/91>Pb2000> Pb1>C44.

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(Received for publication 30 November 2007)