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RADIO-SENSITIVITY OF VARIOUS CHICKPEA GENOTYPES IN M_1 GENERATION II-FIELD STUDIES

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Abstract

The effect of gamma rays and ethylmethane sulphonate (EMS) mutagens on seed germination and plant survival of four chickpea (*Cicer arietinum* L.) genotypes including two desi (Pb2000 and C44), one kabuli (Pb-1) and one desi x kabuli introgression genotype (CH40/91) was investigated in the M_1 generation under field conditions. Seed germination and plant survival decreased in a linear fashion with the increase in gamma irradiation and EMS doses. EMS dose of 0.4% proved most lethal and reduced the percentage of survived plants in desi genotypes Pb2000 and C44 to 68.3% and 62.7%, respectively. In case of kabuli cultivars Pb-1, gamma irradiation of 300Gy and in CH40/91 EMS dose of 0.2 and 0.3% were most lethal doses and reduced the percentage of survived plants to 67.9, 24.4 and 24.2%, respectively. Analysis of variance revealed highly significant differences (P>0.01) among genotypes as well as among different treatment doses regarding plant survival. Highly significant effects of dose x variety interaction for plant survival were observed. From the field experiment, desi type cultivars Pb2000, C44 and kabuli type cultivar Pb-1 appeared to be more radio and EMS resistant whereas CH40/91 was the least radio and EMS resistant.

Introduction

Chickpea (*Cicer arietinum* L.) is the most important food legume of the marginal area of Pakistan. It is one of the main key pulse crop in Indo-Pak subcontinent being used as 'Dal' by majority of the people. 'Desi' chickpea covers nearly 85% area of Thal and 'Kabuli' type around 15% because of its higher susceptibility to various stresses compared to desi type (Akhtar *et al*., 2008; Ali *et al*., 2008; Atta *et al*., 2006; Shah *et al*., 2005; Nadeem *et al*., 2010). The chickpea crop occupied an area of 928.1 thousand hectares with a production of 741.1 thousand tons in Punjab during 2006-07 (Anon., 2007).

Genetic variability in base population becomes indispensable when breeding objectives are more composite. The focal point of a plant breeder is quantitative traits, which are controlled by polygenic interactions. A chain of research work carried out in different countries with various crop species has recognized that physical and chemical mutagens induce polygenic variability (Shah *et al.*, 2008[a]; 2008[b]; Shah *et al.*, 2006; Kharkwal, 1999). Chickpea is a self pollinating crop with narrow variability which is undesirable for any improvement program because genetic variability is a prerequisite for selection of better ideotypes. Among other breeding techniques, induced mutagenesis seems to be a supreme methodology for the induction of desirable genetic variability.

Except physiological damage, all types of mutations may be transferred from M_1 to the subsequent generations (M_2, M_3….). Physiological effects are generally restricted to the M_1 generation and are of varying nature. They represent injuries that can be determined cytologically and measured in an organism or consist of a reaction of the whole organism. Plastidom and plasmon inheritance are related to the functions and properties of the plastid cells, chloroplast and mitochondria. Mutated organelles are in general transferred from generation to generation, mostly via the egg cell. Plastid cells, chloroplast and mitochondria contain DNA and RNA and possible mutations also occur in these organelles' genes. Some properties like cytoplasmic male sterility (CMS) are of fundamental importance in plant breeding. Many workers 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 maturity stage. Reduced germination percentage with increasing doses of gamma irradiation have been reported in chickpea (Haq *et al*., 1992; Toker & Cagirgran, 2004; Khan & Wani, 2005; Toker *et al*., 2005), lentil (Kumar & Sinha, 2003), limabean (Kumar *et al*., 2003) and cowpea (Uma & Salimath, 2001; Gaur *et al*., 2003).

The present study was carried out to obtain practical knowledge about the utilization of physical (gamma irradiation) and chemical (EMS) mutagens in inducing genetic variability in desi (Pb2000, C44), kabuli (Pb-1) and desi x kabuli introgression (CH40/91) chickpea genotypes and to estimate the doses/concentrations of gamma irradiation and EMS appropriate for inducing viable mutations and are effective to reduce growth (e.g. germination, survival and plant height etc.) by a given proportion under field conditions.

Materials and Methods

The seed material used for this study comprised of four chickpea genotypes; two desi (Pb2000 and C44), one kabuli (Pb-1) and one recombinant of desi x kabuli line (CH41/91 or P40/91). The details of seed material, physical and chemical treatments, doses, calculation of radiosensitivity were used as described by Shah *et al*., (2008[a]).

In field experiments the seed treatment procedure for gamma irradiation and ethylmethane sulphonate EMS was same as employed in laboratory experiment (Shah *et al*., 2008[a]). From the LD_{50}/GR_{50} values, obtained from laboratory experiments doses were determined that produced 20-40% inhibition of shoot and root lengths; i.e. a dose of 300 and 400 Gy gamma irradiation and 0.3 and 0.4% of EMS for the desi variety Pb.2000, a dose of 500 and 600 Gy gamma irradiation and 0.3 and 0.4% of EMS for the desi variety C44, a dose of 200 and 300 Gy gamma irradiation and 0.2 and 0.3% of EMS for the kabuli variety Pb.1 and a dose of 200 and 300 Gy gamma irradiation and 0.2 and 0.3% of EMS for desi x kabuli introgression line CH40/91 were applied. One thousand seeds were used per dose for physical and chemical treatments.

The treated and control seeds were grown in experimental plots of NIAB Faisalabad, (situated at Latitude 31^{o} 25' N and 73^{0} 4' 60'E at an altitude of 890 m from sea level in the Punjab province of Pakistan) during winter 2000-2001. Sowing of seeds was done on October 22, 2000 by hand dibbler maintaining one seed per hole. The treated and control seeds were sown on the same day in field. The experiment was laid out in a completely randomized block design with 400 seeds per replication of each genotype for each treatment.

Germination was scored by counting the number of seeds germinated 10 days after sowing. The plant height of 10 randomly selected plants from each treatment was measured at the physiological maturity.

The numbers of surviving plants in each treatment was counted at three growth stages, viz. 20, 60, and 100 days after sowing. At the time of last counting, only those plants were counted as surviving which had pods, good fertility and vigor. All the selected M_1 plants were manually harvested individually on April 22, 2001.

Data was subjected to ANOVA by using MSTATC software (MSTAT-C 1988) and means were compared with Duncan Multiple Range Test (DMRT).

Results and Discussion

The effect of different doses of gamma irradiation and EMS on the plant height in M1 generation of four chickpea genotypes is presented in Table 1(a). Plant height was reduced with the increasing dose of physical and chemical mutagens. The combined analysis for different doses of gamma rays, EMS and genotypes showed that both treatments are effective in reducing the plant height. In Pb2000, Pb1 and CH40/91, the EMS treatments caused more reduction than gamma rays. The overall means of the genotypes and the treatments have been given in Table 1(b). The higher and lower treatments of gamma rays and EMS caused almost same pattern of reduction in plant height. Increase of overall means of varieties, Pb2000 showed maximum plant height (53.49 cm) whereas other genotypes had almost equal plant height. Seed germination and plant survival decreased linearly with the increasing gamma irradiation and EMS doses (Table 2). EMS dose 0.4% proved most lethal dose and reduced the percentage of survived plants in desi genotypes Pb2000 and C44 to 68.3% and 62.7% respectively. In case of kabuli cultivars Pb-1, gamma irradiation 300Gy and in CH40/91 EMS dose of 0.2 and 0.3% were most lethal doses and reduced the percentage of survived plants to 67.9, 24.4 and 24.2% respectively. Reduction in plant survival due to mutagenic treatments is a common phenomenon in induced mutation experiments (Haq *et al*., 1992; Toker & Cagirgan, 2004; Khan & Wani, 2005; Toker *et al*., 2005). Analysis of variance revealed highly significant differences (p>0.01) among genotypes as well as among different treatment doses regarding plant survival (Table 3). Highly significant effects of dose x variety interaction for plant survival were observed. The number of surviving plants and plant height linearly decreased with the increase in growth duration in mutagen and EMS treated populations. Similar results have been reported in rice (Katoch *et al.,* 1992; Sarawgi & Soni, 1993; Cheema & Atta, 2003). Apparently, reduction in plant height could be due to the inhibition of DNA synthesis or other physiological damage after mutagenic treatments. The retardation of plant height is also considered to depend upon several phenomena such as growth-factor destruction, transport inhibition or production of diffusible growth-retarding substances (Sjodin, 1962). Micke & Wohrmann, (1960) have reported that there are critical phases during plant development at which lethal effects are more prominent. The maximum reduction in plant height has been reported with gamma irradiation treatments in *Vigna Sesquipedalis* (Kon *et al*., 2007) and chickpea (Khan *et al*., 2000; Toker & Cagirgan, 2004; Khan & Wani, 2005; Toker *et al*., 2005). It may be mentioned that gamma irradiation doses of 300-400Gy and EMS doses of 0.3-0.4% were selected for desi type genotypes and gamma irradiation doses of 200-300Gy and EMS doses of 0.2-0.3% for kabuli and desi x kabuli introgression genotypes which were effective in causing 20-40% reduction in shoot and or root length of seedlings under the laboratory conditions (Shah *et al*., 2008[a]). However these doses drastically decreased the number of surviving plants in the field, which was partly due to the adverse soil and climatic conditions prevailing during the vegetation period of M_1 material, which could lead to a great variability of the survival rate as pointed out by Gaul (1963).

Table 1. Effect of different doses of gamma irradiation and ethylmethane sulphonate (EMS) on plant height in M_1 generation of four chickpea genotypes.

(a) Two way tables of means

Genotype	Treatment				
	Control	Gamma irradiation		EMS (%)	
		Higher dose	Lower dose	Higher dose	Lower dose
Pb2000	66.13	54.47	47.87	52.53	46.47
C44	61.27	47.40	42.80	51.53	45.87
Pb-1	60.33	50.13	45.53	49.27	44.67
CH40/91	62.13	50.20	44.47	48.93	43.93

SE ± 3.522

(b) One way tables of means

Genotype	Pb2000	C44	Pb-1	CH40/91
Means	53.49	49.77	49.99	49.93

SE ± 1.575

Means	Control	Treatment			
		Gamma irradiation		EMS (%)	
		Higher dose	Lower dose	Higher dose	Lower dose
	62.47	50.55	45.17	50.57	45.23

SE ± 1.761

Table 2. Seed germination and plant survival at three growth stages in M_1 generation of four chickpea genotypes treated with different doses of gamma irradiation and EMS.

Genotype	Dose	No. of surviving plants at 10th day	No. of surviving plants at 20th day	No. of surviving plants at 60th day	Survival % of control
Pb.2000	**Control**	55.00 ± 1.67	60.00 ± 2.50	51.67 ± 4.17	
	300Gy	41.52 ± 4.40	44.75 ± 1.62	32.72 ± 6.02	71.4
	400Gy	43.92 ± 2.25	45.95 ± 1.02	29.43 ± 3.26	71.6
	0.3% EMS	44.88 ± 3.04	48.31 ± 1.72	26.81 ± 3.75	72.0
	0.4% EMS	40.63 ± 4.91	42.44 ± 0.91	30.81 ± 5.82	68.3
C44	**Control**	70.41 ± 2.29	66.67 ± 1.87	65.83 ± 0.42	
	500Gy	47.34 ± 1.05	46.14 ± 0.60	45.25 ± 0.45	68.4
	600Gy	47.97 ± 1.49	47.03 ± 0.47	45.00 ± 1.02	69.0
	0.3% EMS	44.44 ± 0.07	43.31 ± 0.57	44.31 ± 0.50	65.1
	0.4% EMS	42.06 ± 0.13	43.31 ± 0.63	41.81 ± 0.75	62.7
Pb.1	**Control**	70.42 ± 1.05	60.42 ± 5.00	58.33 ± 1.05	
	200Gy	53.04 ± 2.60	49.11 ± 1.97	47.85 ± 0.63	79.3
	300Gy	44.37 ± 1.17	42.03 ± 1.17	42.03 ± 0.00	67.9
	0.2% EMS	54.13 ± 0.41	57.06 ± 1.47	54.94 ± 1.06	87.8
	0.3% EMS	45.31 ± 0.54	48.25 ± 1.47	46.38 ± 0.94	74.0
CH40/91	**Control**	50.42 ± 1.05	50.00 ± 0.21	48.33 ± 0.84	
	200Gy	42.5 ± 2.59	46.28 ± 1.89	37.32 ± 4.48	84.8
	300Gy	28.06 ± 1.78	33.75 ± 2.85	24.50 ± 4.63	58.0
	0.2% EMS	13.69 ± 2.94	14.75 ± 0.53	7.81 ± 3.47	24.4
	0.3% EMS	13.69 ± 3.47	15.50 ± 0.91	6.75 ± 4.38	24.2

Table 3. Analysis of variance for the effect of different doses of gamma irradiation and EMS on plant survival in M_1 generation of four chickpea genotypes in the field.

Source of variation	df	Sum of squares	Mean squares	P -value
Replications	2	14.566	7.283	0.0256*
Main effects				
Dose	4	4669.889	1167.472	0.000***
Variety	3	6339.199	2113.0665	0.000***
Interaction				
Dose x variety	12	1997.37	166.448	0.000***
Error	38	68.449	1.801	

* Significant at 5% level

** Highly significant at 1% level

From the field experiment, desi type cultivars Pb2000, C44 and kabuli type cultivar Pb-1 appeared more radio and EMS resistant whereas CH40/91 was the least radio and EMS resistant, which confirmed the findings of laboratory experiment (Shah *et al*., 2008[a]). Hence for genotypes like CH40/91 (Introgression line of desi x kabuli) should be treated with lower doses of physical and chemical mutagens for obtaining positive and useful mutations.

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