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Changes in the Biological Effects of Gamma Irradiation with Gibberellic Acid in M_2 Generation of Chickpea (*Cicer arietinum* L.)

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Abstract: Seeds of 250 plants from M_1 generation of three chickpea genotypes viz, Noor 91 (white), Punjab 91 (brown) and C 141 (black) at 40, 50 and 60 Kr separately and with gibberellic acid (GA_3) along with control were grown to raise the M_2 generation. The effects on 100-seed weight, grain yield, biological yield, harvest index, days to flowering and maturity in M_2 generation were highly significant ($p<0.01$) within genotypes, treatments and also for their interaction. Statistically significant increase in 100-seed weight was observed with the combine treatment at 40 Kr while it was decreased at 60 Kr as compared with gamma irradiation. Grain yield was significantly increased with gamma irradiation however, stimulation was recorded with the application of GA_3. Biological yield was decreased while, harvest index was increased with both types of treatments. However, more harvest index was recorded with combine treatment. Days to flowering were increased at 40 and 60 Kr with gamma irradiation while, decreased at 40 and 50 Kr with the combine treatment. Days to maturity were decreased with both the treatments.

Key words: Gamma irradiation, gibberellic acid, modulation, yield, chickpea

Introduction

In recent years mutation breeding has gaining ground for inducing genetic changes and creation of new genetic resources (Awan, 1999). The improvement of a crop depends to a large extent on the genetic variability as it provides us the raw material for selection of better genotypes. Since, greater the genetic diversity in the base. population, wider would be the scope of selection. The primary objectives of mutation breeding are to enhance mutation frequency, widen the mutation spectrum and realize directed mutagenesis. It is well established that mutagenicagentsareeffectiveforinducing genetical changes intreated population (Kasim *et al.,* 1977; Shakoor *et al*., 1978a, 1978b; Kalia and Gupta, 1988a, 1988b).

Little natural variability is found in chickpea for conspicuous morphological and physiological characters. Several workers have attempted for induction of mutation using either physical or chemical mutagens for evolving new genotypes (Kharkwal *et al.,* 1988; Haq *et al.,* 1989; Hassan and Khan, 1991; Shamsuzzaman and Shaik, 1991), Radiation, therefore, appears to be a useful tool in plant breeding and genetics.

The extent of genetic variability is more important than the total variability. Gamma irradiation in combination with other chemical mutagens is applied for, widening the frequency and mutation spectrum for extra genetic variability. Gibberellic acid serves manifold growth related functions in plants by enhancing replication, transcription and different enzymatic systems. (Callebaut *et al.,* 1980; Uppal and Maherchandani, 1988; Zhebrak, 1989; Ali and Ansari, 1989; Arora *et al.,* 1989). Effect of gamma radiation is changed with the radio protective effect of gibberellic acid. It has been established that the impaired growth due to gamma irradiation can be restored by exogenous application of gibberellic acid. It may be possible that gibberellic acid may modulate the effects of gamma irradiation. It brightens the scope for increasing both the frequency and spectrum of mutation. There is a dearth of knowledge on the modulation of radiosensitivity with gibberellic acid for the segregating populations as only study in M_1 generation was reported (Khan *et al.,* 2000). Therefore, it was planned to determine the effectiveness of gamma irradiation and efficiency of gibberellic acid to modulate the radio sensitivity for various yield characters.

Materials and Methods

Dry seeds were exposed to gamma irradiation at doses of 10, 20, 30, 40, 50, 60, 70, 90 and 110 Kr to 1000 seeds for each treatment in three genotypes at Nuclear Institute for Food and Agriculture (NIFA), Peshawar. On the basis of seedling performance doses of 40, 50 and 60 Kr were selected for inducing genetic variability on large scale. A part of the irradiated seeds after one hour of soaking under continuous aeration were subjected to 0.5 mM aqueous solution of gibberellic acid for 16 hours with constant shaking. Non irradiated seeds soaked in water were kept as control in the case. After treatment seeds were washed in running tap water and then were dried on blotting paper. Treated along with control seeds were sown in split plot design with three replications at Barani Agriculture Research Institute (BARI) Chakwal in 1995 to raise the M_1 generation. Seeds of 250 plants were separately collected and were sown in the next year in a split plot design to raise the M_2 generation while, from control population seeds were bulked. Data on 40 randomly selected plants was appropriately recorded for various characters.

Results and Discussion

In this generation various variations were obtained due to the genetical changes preserved in the plants of M_1 generation and lasting throughout the developmental period. These genetic variations are manifested as induced variants, which provides the raw material for selection of desirable genotypes for crop improvement and also to widen the germplasm pool. Data on various plant characters were recorded and expressed as follows.

100-Seed weight (g): The analysis of variance for the effect of different doses of gamma irradiation with and without the application of gibberellic acid on 100-seed weight per plant in M_2 population of chickpea (Table 1) indicates highly significant ($p<0.01$) variation within genotypes and treatments. Variety-treatment interaction was also highly significant ($p<0.01$). It reflects that a marked variability is induced for this character across the different treatments. In the previous research, similar findings have also been reported by Sarma *et al.* (1991) in green gram. Charumathi *et al.* (1992) in black gram and Gupta *et al*. (1996) in horse gram. Maximum 100-seed weight 26.19 g per plant was observed in C141 (Table 2), followed by 25.72 and 25.37 g per plant in Punjab 91 and Noor 91, respectively.

100-seed weight was differentially responded to both mutagen treatments. Gamma irradiation decreased the 100-seed weight significantly ($p<0.01$) at 60 Kr as compared to control. Application of gibberellic acid modulated the effects of gamma irradiation and 100-seed weight was increased at all irradiation dosages. There was significant ($p<0.01$) increase in 100-seed weight at 40 Kr, while at 50 and 60 Kr doses the increase in 100-seed weight was non-significant as compared to control.

The varieties responded differentially for 100-seed weight to the two mutagenic treatments. In Noor 91 the 100-seed weight decreased non-significantly at all gamma irradiation dosages as compared to control. However, with the application of GA_3 100-seed weight increased significantly ($p<0.01$) at 40 Kr treatment. 100-seed weight in Punjab 91 increased non-significantly at 40 and 50 Kr while, it was decreased significantly ($p<0.01$) at 60 Kr with gamma irradiation as compared to control. Application of gibberellic acid decreased the 100-seed weight at 40 and 60 Kr doses, while at 50 Kr treatment it was increased as compared to control. However, the decrease or increase in 100-seed weight was non-significant as compared to control. Gamma irradiation in C141 decreased the 100-seed weight non-significantly at 40 and 50 Kr but significantly ($p<0.01$) at 60 Kr treatment as compared to control. 100-seed weight increased significantly ($p<0.01$) with GA_3 at 40 and 60 Kr treatment, while the increase at 50 Kr was non-significant as compared to control.

Grain yield per plant (g): Highly significant difference was recorded within treatments and genotypes for the effect of different doses of gamma irradiation separately and with the application of gibberellic acid on grain yield per plant in M_2 population of chickpea. The interaction between genotype and treatment was also highly significant ($p<0.01$). It reflects the highly inconsistent performance of genotypes for this character. Previously, with gamma irradiation similar observation have been reported by Sarma *et al.* (1991) and Charumathi *et al.* (1992). Maximum grain yield of 37.00 g per plant was observed in Punjab 91 (Table 2), followed by 25.42 and 25.21 g per plant in Noor 91 and C 141, respectively.

It is seen from the results that the grain yield increased significantly ($p<0.01$) across the different mutagenic treatments of gamma irradiation and with gibberellic acid as compared to control. Gibberellic acid increased the grain yield by modulating the effects of gamma irradiation and maximum increase in grain yield was observed at 50 Kr treatment.

A differential response of varieties across the various mutagenic treatments was observed. In Noor 91 grain yield per plant increased significantly ($p<0.01$) with both mutagenic treatments. However, with the application of gibberellic acid significantly more grain yield was recorded at 40 and 50 Kr. Grain yield in Punjab 91 decreased significantly ($p<0.01$) with gamma irradiation at 60 Kr treatment as compared to control. Application of gibberellic acid changed the effect of gamma irradiation and significant ($p<0.01$) decrease and increase in grain yield was observed at 40 Kr and 50 Kr treatment, respectively as compared to control. In C141 gamma irradiation had non-significant effects on grain yield while, with gibberellic acid at 50 and 60 Kr significant ($p<0.01$) increase was observed as compared to control.

Biological yield (g): The analysis of variance for the effect of different doses of gamma irradiation with and without the application of gibberellic acid on biological yield per plant in M_2 population of chickpea (Table 1) shows highly significant ($p<0.01$) differences among genotypes and treatments as well as for their interaction. It indicates highly inconsistent performance of genotypes across various treatments. Genotype Punjab 91 exhibited the maximum biological yield 99.00 g per plant as compared with 97.94 and 89.21 g per plant in 0141 and Noor 91, respectively (Table 2).

It is apparent from the results that the different mutagenic treatments decreased the biological yield significantly ($p<0.01$) as compared to control. Application of gibberellic acid decreased the biological yield gradually with an increase in gamma irradiation dosages however, with gamma irradiation the response was inconsistent.

A differential response of genotypes for biological yield (Table 2) was observed across the different mutagenic treatments. In Noor 91 the biological yield decreased non-significantly with gamma irradiation while, significant ($p<0.01$) decrease was observed with gibberellic acid at 50 and 60 Kr treatments as compared to control. Punjab 91 exhibited significant decrease in biological yield with gamma radiation at 40 and 60 Kr treatments. Whereas, a regular and non-significant decrease in biological yield was observed across the various treatments with gibberellic acid as compared to control. GA_3 treatment changed the effects of gamma irradiation and biological yield increased at 40 Kr. In C141 biological yield decreased significantly ($p<0.01$) and consistently across the two mutagenic treatments as compared to control.

Harvest index (%): The analysis of variance for the effect of different doses of gamma radiation separately and with the treatment of gibberellic acid on harvest index per plant in M_2 population of chickpea (Table 1) indicates highly significant ($p<0.01$) variation among the treatments and genotypes. Genotype-treatment interaction was also highly significant ($p<0.01$). This indicates that genotypes responded differently for this character across the various treatments. Kalia and Gupta (1988b) reported sufficient variation in harvest index induced due to gamma irradiation in M_2 population of lentil.

Punjab 91 exhibited a maximum harvest index of 37.43% per plant, followed by 25.94% and 25.57 % per plant in C141 and Noor 91, respectively (Table 2).

It is evident from the results that the mutagenic treatment increased the harvest index significantly ($p<0.01$) as compared to control, but the effects were inconsistent in

Table 1: Mean of squares for different characters of M_2 generation in chickpea genotypes

Variables	D.F	100-S Weight	Grain Yield	Biological Yield	Harvest Index	Days to Flowering	Days to Maturity
Replicates	2	0.01	0.58	1.80	0.13	1.44	0.78
Varieties (V)	2	3.55**	956.47**	606.52**	760.92**	221.39**	75.27**
Error a	4	0.04	0.08	0.96	0.42	0.01	1.07
Treatments (T)	6	0.83**	25.74**	200.63**	59.70**	11.40**	17.81**
T×V	12	0.29**	4.87**	46.86**	14.26**	7.41**	18.41**
Error b	36	0.04	0.43	1.23	0.68	0.48	0.64
Total	62						

**Highly significant at 0.01 probability level

Table 2: Effect of gamma irradiation separately and with gibberellic acid on various characters in M_2 generation of three chickpea genotypes

Variables	Varieties	Control	Gamma irradiation			Gamma irradiation+GA_3 (0.5 mM)			
			40 Kr	50 Kr	60 Kr	40+GA_3	50+GA_3	60+ GA_3	Mean
	Noor 91	25.22 b	25.10 b	25.20 b	25.07 b	26.30 a	25.36 b	25.32 b	25.37 c
100-S Weight	Punjab 91	25.82 ab	25.95ad	25.86 ab	25.17 c	25.70 ab	26.00 a	25.52 bc	25.72 b
(1)	C 141	26.03 b	25.97 b	26.07 b	25.42 c	26.67a	26.40 ab	26.75 a	26.19 a
	Mean	25.70 b	25.67 b	25.71 b	25.22 c	26.22 a	25.92 b	25.86 b	
	Noor 91	19.80 d	25.96 bc	24.93 c	26.45 b	29.27 a	26.76 b	24.88 c	25.42 b
Grain Yield	Punjab9l	37.56 bc	36.27 c	37.50 bc	34.60 d	33.92 d	41.26 a	37.91 b	37.00 a
(2)	C 141	23.35 c	24.42 bc	24.63 bc	24.04 bc	24.75 bc	29.50 a	25.77 b	25.21 b
	Mean	26.90 d	28.85 bc	29.02 be	28.36 c	29.31 b	32.50 a	29.52 b	
	Noor 91	94.71 a	89.22 ab	88.37 ab	88.87 ab	93.02 ab	84.90 b	85.37 b	89.21 b
Biological Yield	Punjab9l	105.48 a	91.30 c	101.60 a	93.75 bc	102.88 a	100.57ab	97.45abc	99.00 a
(3)	C 141	112.57 a	98.77 b	96.15 b	92.75 bc	99.20 b	98.57 b	87.58 c	97.94 a
	Mean	104.25 a	93.10 cd	95.37 bc	91.80 c	98.37 h	94.68 bcd	90.13 d	
	Noor 91	20.90 c	29.00 b	28.22 b	29.80 ab	31.46 a	31.50 a	29.14 b	25.57 b
Harvest Index	Punjab9l	35.61 c	39.72 ab	36.90 c	36.91c	32.97d	41.02 a	38.90 b	37.43 a
(4)	C 141	21.06 c	24.72 b	25.60 b	25.91 b	24.95 b	29.93 a	29.43 a	25.94 b
	Mean	25.86 e	31.15 c	30.24 cd	30.87 cd	29.80 d	34.15 a	32.5 b	
	Noor 91	120.00 a	120.50 a	118.50 b	120.00 a	118.00 a	118.50 b	120.00 a	119.35 b
Days to Flowering	Punjab 91	114.50 a	114.00 a	115.00 a	114.50 a	114.00 a	114.50a	111.50b	114.00c
(5)	C 141	119.50b	122.50a	119.50b	123.50a	117.50c	116.50c	120.00b	119.85a
	Mean	118.00 b	119.00 a	117.6.6 b	119.00a	116.00 c	116.50 c	117.16 b	
	Noor 91	166.50 a	165.00 a	167.50 a	164.00 a	161.50 b	164.00 a	167.50 a	165.14 c
Days to Maturity	Punjab 91	169.50 a	165.50hc	168.50ab	169.50 a	164.50bc	164.00c	168.00 ab	167.07b
(6)	C 141	170.50 a	172.50 a	166.50bc	169.50ab	167.00bc	171.50 a	165.00 c	168.92 a
	Mean	168.83 a	167.66ab	167.50ab	167.66ab	164.33 c	166.50 b	166.83ab	
		1	2	3		5	6		
Sx varieties (V)		0.0436	0.0617	0.2138	0.1414	0.0218	0.2257		
Sx treatments (T)		0.0666	0.2187	0.3696	0.2748	0.2309	0.2666		
Sx VXT		0.1154	0.3785	0.6.034	0.4760	0.4000	0.4618		

the two treatments. Maximum harvest index of 34.15 per plant was observed with gibberellic acid at 50 Kr treatment against 25.86 per plant in control.

Varieties varied in their response to various treatments. In Noor 91 harvest index was increased significantly ($p<0.01$) across all the mutagenic treatments as compared to control. However, significantly more harvest index was observed at 40 and 50 Kr with the application of gibberellic acid. In Punjab 91 harvest index increased non-significantly with gamma radiation except at 40 Kr. Application of gibberellic acid decreased the harvest index significantly ($p<0.01$) at 40 Kr while, increased significantly ($p<0.01$) at 50 and 60 Kr treatment as compared to control. C 141 exhibited a significant ($p<0.01$) increase with both mutagenic treatments. However, significantly more harvest index was obtained at 50 and 60 Kr with the treatment of gibberellic acid.

Days to flowering: The analysis of variance for the effect of different doses of gamma irradiation with and without the application of gibberellic acid on days to 50% flowering in M_2 population of chickpea (Table 1) indicates highly significant ($p<0.01$) differences within the genotypes and treatments. The interaction between genotype-treatment was also highly significant ($p<0.01$). It reflects highly inconsistent performance of genotypes for this character. Maximum time taken to 50% flowering was 119.85 days in C141 followed by 119.35 and 114.00 days in Noor 91 and Punjab 91, respectively (Table 2). It is apparent from the results that the mutagenic effect on time to 50% flowering was different across the two mutagenic treatments. A significant ($p<0.01$) increase in number of days to 50% flowering was observed with gamma irradiation at 40 and 60 Kr treatment as compared to control. However, the time taken to 50% flowering with gibberellic acid treatments was significantly ($p<0.01$) less as compared to control at 40 and 50 Kr treatments.

The response of varieties towards the two mutagenic treatments varied. In Noor 91 the effect of gamma irradiation on time to 50% flowering was erratic and less time to 50% flowering was observed at 50 Kr treatment as compared to control. The application of gibberellic acid significantly decreased the time to 50% flowering as compared to control at 40 and 50 Kr treatments. In Punjab 91 similar response to the two mutagenic treatments was observed. Days taken to 50% flowering were affected non-significantly across the various mutagenic treatments except at 60 Kr with GA_3 treatment a significant ($p<0.01$)

decrease as compared to control was observed. In C141 the days to 50% flowering were significantly ($p<0.01$) increased at 40 and 60 Kr treatments with gamma irradiation as compared to control. However, the application of gibberellic acid decreased the time to 50% flowering at 40 and 50 Kr treatments as compared to control.

Days to maturity: The results indicate highly significant differences within treatments and genotypes. The genotype-treatment interaction was also highly significant ($p<0.01$). It reflects that a marked variation in genetic spectrum obtained at different doses. Maximum time taken to maturity was 168.92 days in C141 followed by 167.07 and 165.14 days in Punjab 91 and Noor 91, respectively (Table 2). It is apparent from the results that the maturity was responded differentially to the two mutagenic treatments. The effects of gamma radiation at all levels on maturity was non-significant, while the number of days to maturity with gibberellic acid at 40 and 50 Kr treatment were significantly ($p<0.01$) less as compared to control. A differential response of maturity among various treatments was observed for the varieties. In Noor 91 the time taken to crop maturity was non-significant at various mutagenic treatments except at 40 Kr treatment with gibberellic acid where a significant ($p<0.01$) decrease was observed as compared to control. In Punjab 91 significant ($p<0.01$) decrease in time taken to maturity was observed at 40 Kr treatment with gamma irradiation, while with gibberellic acid at 40 and 50 Kr treatment as compared to control. C141 exhibited significant ($p<0.01$) decrease in time to maturity, at 50 Kr treatment with gamma irradiation, while with gibberellic acid significant ($p<0.01$) decrease in days to maturity was found at 40 and 60 Kr treatment as compared to control.

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