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Effect of Gamma Irradiation on Wheat Immature Culture Regenerated Plants

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Abstract: The effect of gamma rays on grains of wheat (*Triticum aestivum* L.) irradiated with 150, 250, 350 and 450 Gy was studied on second generation (M_2) plants immature embryos culture callus growth rate, plant regeneration, agronomic traits and phenotypic and genotypic coefficients of four wheat cultivars. The results showed a highly significant effect between the four tested wheat cultivars i.e. Sids-1, Sakha-93, Sahel-1 and Giza-168 under different doses of gamma rays. The low dose of 150 Gy seemed to have a stimulating effect on all characters studied. On the other hand, the high doses caused significant reduction as compared to the control for all studied traits. The highest percentage of callus growth rate and plant height was obtained in Sakha-93. The interaction between gamma rays at 150 Gy and Sids-1 give a significant increase in callus induction percentage, plant regeneration, all growth characters, yield and its component as compared to control. Genotypic and phenotypes correlation were positive and highly significant between callus growth rate and grain yield per plant.

Key words: Wheat cultivars, gamma rays, immature embryos, agronomic traits

INTRODUCTION

Wheat is one of the most important cereal crop used in human food and animal feed in Egypt and overall the world. Recently, a great attention of several investigators has been directed to increase productivity of wheat per unit area by introducing more productive varieties or improving agricultural practices.

Gamma rays played a great role in inducing genetic changes in DNA in different crop plants. It seems that the sensitivity to gamma rays differ from wheat variety to another. Therefore, the experiments must be conducted to detect the radio-sensitivity of different developed cultivars Azer[1]. Amin[2] found that irradiation of wheat grains with 2-4 K-rad caused increases in vegetative growth characters. Kassem and Naser[3] found that in second generation (M_2), the 25K-rad dose increased the values of phenotypic and genotypic coefficients of variation as well as heritability for most studied characters of the two wheat varieties comparing with 20 and 15 K-rad doses. Rashed *et al*[4] reported that callus fresh weight, days for callus initiation, callus growth rate and plant regeneration in relation to the average number of callus were affected in the four maize inbred lines at most of gamma rays doses. Abdel-Hady and Abou-Deif[5] showed that 100 Gy had a significant increase in callus induction and plantlets formation in the three maize inbred lines. However, the high doses showed highly significant reduction in the same characters.

Abdel-Hady and Ahmed[6] reported that low dose of 150 Gy seemed to have a stimulating effect on agronomic of four wheat cultivars in the first mutagenic generation (M_1). Moreover, the electrophoretic patterns indicated that the presence of wide genetic variations between the gamma doses and the control.

The present study aims to throw light on the response of different irradiated wheat cultivars (*Triticum aestivum* L.) with regard to immature embryo culture, regeneration plants, and magnitude of phenotypic and genotypic coefficients of variation growth characters, yield and its attributes in second generation (M_2).

MATERIALS AND METHODS

This study was carried out in Tissue Culture Laboratory of Botany Department, National Research Center, Dokki, Cairo, Egypt. Field experiment was carried out at the Agricultural Experimental station, National Research Centre at Shalakan, Kaluobia Governorate.

A. Plant material: Seeds of four bread wheat (*Triticum aestivum* L.) cultivars; Sids-1, Sakha-93, Sahel-1 and Giza-168 were irradiated with four doses of gamma rays (150, 250, 350 and 450 Gy). at National Center for Radiation and Technology, Cairo, Egypt.

b. Methods: From first generation (M_1) plants were taken at random from experimental unit. Grains of 5 plants of each treatment were bulked and sown to obtain second generation (M_2). Random samples of twenty individual plants were taken from every treatment at harvest in M_2 estimate the following characters: Plant height (cm),

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number of tillers per plant, number of spikes per plant, 1000-grains weight (g), grain yield per plant (g) and grain yield (ton/feddan).

Tissue culture experiment: Callus cultures of four bread wheat (M_2) were induced from immature embryos (13-16 days after anthesis) as indicated by[7]. The caryopses were removed fromthe spike, surface-sterilized for 5 min. in 5% chlorox (sodium hypochorite) mixed with two drops of TWeen 20 and washed in sterile distilled water. Fifty embryos of each genotypes were planted on a solid agar medium with the scutellar protein of the embryo. Murashige and Skoog[8] inorganic components and B5-vitamins (Gamborg *et al*)[9] were used. It was supplemented with 0.5 mg/l thiamine-HCl, 150 mg/l asparagine 30 g/l sucrose, 100 mg/l inositol., 8 g/l agar and 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) in ten replicates (jars) with a frequency of four embryos per jar for callus induction.

The previous mentioned medium was used for five times subculturing with an intervalperiod of seven days. After two weeks, all calli were transferred to the same medium except the concentration of 2, 4-D as decreased to 0.5 mg/l (Shooting medium) and free-hormone medium for root formation. All media were adjusted at pH 5.8 and autoclaved for 20 min, the cultures used at the former stages were kept at 26°C and 16/8 hours day light and dark,, respectively. The percentage of callus induction defined as the embryos forming callus over the total number of embryos. Callus growth rate was calculated according(10). Percentage of plantlets formation was calculated. The experimental design was complete randomized blocks. Analysis of variance and L.S.D. values estimated according Bhaskaran *et al*[11]. Genotypic and phenotypic coefficients of variation between callus induction, callus growth rate and grain yield/plant.

RESULTS AND DISCUSSIONS

I-Effect of gamma irradiation on tissue cultured and regenerated plants:

1. Callus induction: Data presented in Table 1 and Fig. 1 showed the percentage of callus induction of the four tested genotypes of wheat (M_2) from immature embryos treated with various doses of gamma rays (0, 150, 250, 350 and 450 Gy). The data also showed a highly significant effect between the four tested wheat genotypes under different doses of gamma rays. There was a surpassing response for Sids-1 which gave the highest percentage (74.48%) of callus induction, however Giza-168 gave the lowest one (59.60%). Therefore it could be concluded that genotypes differedin their ability to different doses ofgamma raysdepending on the genetic make up of callus. The average of callus induction at 150 Gy showed highly significantly increase (88.99%), whereas the high reduction (45.98% and 28.65%) was obtained with gamma rays treatments at 350 and 450 Gy respectively as compared to control.

There was a markedly increase in callus induction percentage at 250 Gy was recorded, however such increasewas not enough to reach the levelofsignificance as compared to control.

Withregard to theinteractionbetween genotypes and gamma-ray doses, there were highly significant differences between all treatments as shown in Table 1. Highly significant increaseon callusinduction percentage was observed between the fourtestwheat genotypes and gamma rays at 150 Gy. However, the increase at treatment with gamma rays of 250 Gy,could not reach the level of significant as compared to control. Whereas, at 350 and 450 Gy scorded highly significant decreased in callus induction of the four test wheat genotypes.

It could be concluded that the lowdose of gamma-ray caused increase in callus induction, while higher doses reduced it. Similarresults obtained by Rashed *et al*[4] and Abdel-Hady and Abou-Deif[5].

2. Callus growth rate: Date in Table 2 showed that, the callus growth rate varied significantly in the four tested wheatgenotypes in the second generation (M_2). Sakha-93 and Sids-1 surpassed (with mean of 198.33 % and 218.80%), while both Sahel-1 and Giza-168 genotypes were sensitive to gamma rays (with mean of178.87% and 149%) respectively. This means that the final biomass of callus produced from the immature embryo culture of wheat genotypes mainly depending on the genotype response. This result could be due to the effect of the endogenous hormones produced in theseembryos which is adjusted by the gene action in this regard. Bhaskaran and Smith[12] reported that meristematic cells fromtissues are the target for plant growth regulator action.

The highest growth rate of calli was obtained at the lower dose of gamma ray 150 Gy which showed 15.22% increase as compared to the control. However, reduction in calli growth rate were obtained in calli produced after treatments with 250Gy, 350 Gy and 450 Gy of gamma rays which recorded 26.18%, 57.30% and 85.98% respectively as compared to the control.

Gamma rays treatment at 150 Gy gave highly significant difference between callus growth rate and the controlin the four tested wheat genotypes 16.14%, 17.31, 15.61 and 11.10%. Therefore an increase in callus growth rate were resulted in Sakha-93, Sids-1, Sahel-1 and Giza-168 respectively as compared to their controls. On the contrary, induced calli after treatment with 250Gy, 350 Gy and 450 Gy showed highly significantly reduction in callus growth rates as compared with their controls. From these results, it is clearthat the reduction in callus growth rate showed a gradual decrease with increasing gamma-ray doses from 150 Gy to 450 Gy as compared to control.

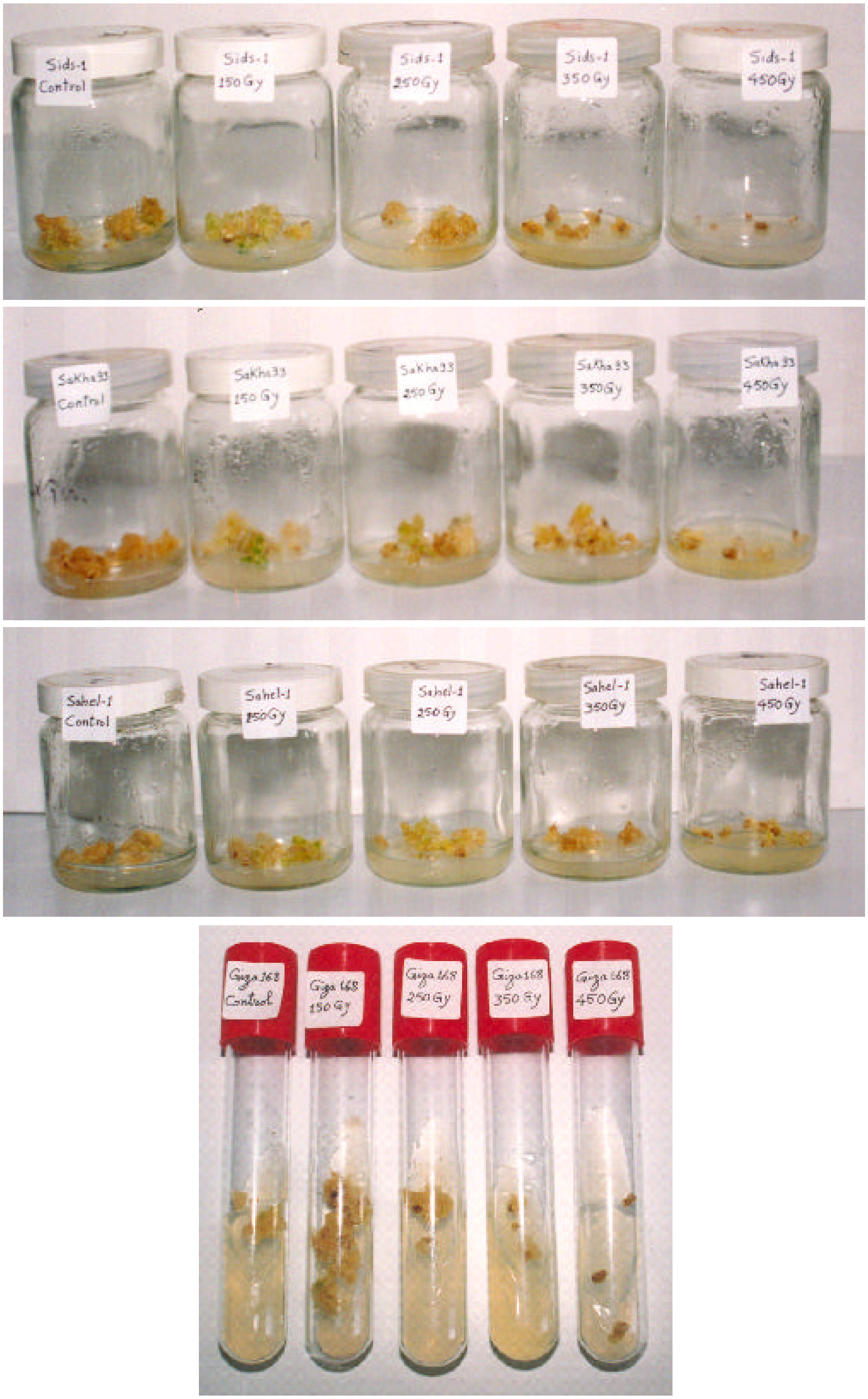

Fig. 1: Effect of gamma rays on callus induction (%) in (M_2) generation of the four wheat genotypes.

Table 1: Effect of gamma rays on callus induction (%) in (M_2) generation of the four wheat genotypes.

Wheat genotypes	Gamma ray doses (Gy)					Genotype means
	0	150	250	350	450	
Sids-1	88.33	93.36**	90.32**	59.17**	41.21**	74.48
Sakha-93	85.10	90.10**	86.41**	52.72**	33.11**	69.49
Sahel-1	83.27	87.21**	84.16**	39.84**	22.17**	63.23
Giza-168	80.63	85.30**	81.27**	32.70**	18.12**	59.60
Means	84.33	88.99	85.54	45.98	28.65	
L.S.D.	5%	1%				
Genotype (G)	1.344	1.80				
Gamma doses (r)	1.203	1.61				
G x ?	2.689	3.60				

Table 2: Effect of gamma rays on callus growth rate in M_2 generation of the four wheat genotypes.

Wheat genotypes	Gamma ray doses (Gy)					Genotype means
	0	150	250	350	450	
Sids-1	279.33	327.67**	216.67**	126.33**	41.67**	198.33
Sakha-93	295.53	343.00**	244.33**	151.33**	60.33**	218.80
Sahel-1	262.67	303.67**	193.67**	103.00**	31.33**	178.87
Giza-168	240.33	267.00**	140.67**	79.33**	17.67**	149.00
Means	269.33	310.33	198.83	115.00	37.75	
L.S.D.	5%	1%				
Genotypes (G)	2.789	3.730				
Gamma rays (g)	2.495	3.340				
G x ?	5.579	7.460				

Table 3: Effect of gamma rays on plant regeneration (%) in (M_2) generation of the four wheat genotypes.

Wheat genotypes	Gamma ray doses (Gy)					Genotype means
	0	150	250	350	450	
Sids-1	65.31	81.82**	73.56**	57.11**	35.17**	62.59
Sakha-93	60.25	75.78**	66.71**	48.20**	23.13**	54.81
Sahel-1	53.12	65.91**	58.38**	31.72**	6.71**	43.17
Giza-168	50.14	63.39**	53.64**	23.33**	2.19**	38.54
Means	57.21	71.73	63.07	40.09	16.80	
L.S.D.	5%	1%				
Genotypes (G)	5.668	7.58				
Gamma rays (g)	5.069	6.78				
G x ?	11.355	15.17				

This reduction is due to the inhibiting effect of high doses (350 Gy and 450 Gy) of gamma-ray, while 150 Gy stimulated callus growth rate in all studied wheat genotypes. Similar results were obtained by[13,14,4,5]. Also, Amin[2] found that irradiation of wheat grains with 2-4 K-rad caused increases in vegetative growth characters.

3. Plant regeneration: Plant formation from the induced calli differed highly significantly on the basis of the produced callus percentage except between Sahel-1 and Giza-168 Table 3. The highest plant formation percentage (62.59%) was obtained in Sids-1, while the lowest one occurred in Giza-168 (38.54%). This indicates that, the plantlet production was mainly depended on the variation between different genotype, which could be reflected on the amount of growth substances produced in the formed callus and enhanced plant formation and development

Differentiation of plants from calli derived from irradiated wheat grains varied according to the does used of gamma –ray Table 3. Therefore, a highly significant (25.38%) and significant increase (10.24%) was obtained at gomma a rays doses of 150 and 250 Gy respectively as compared to their control. On the contrary, plant regeneration percentage after treatment with 350 Gy and

Table 4: Estimates of genotypic and phenotypic correlation among studied characters. (values of genotypic correlation are above the diagonal and those phenotypic correlation are below the diagonal).

Characters	Gamma doses (Gy)				
	0	150	250	350	450
Callus induction x callus growth rate	1.039	0.999	1.022	1.003	0.998
	0.876	0.864	0.847	0.988	0.985
Callus induction x grain yield/plant	146.564	1.564	1.362	1.020	1.001
	0.519	0.726	0.652	0.925	0.984
Callus growth rate x grain yield/plant	148.613	1.590	1.229	0.997	0.990
	0.497	0.638	0.724	0.935	0.957

450 Gy. Gamma-ray were highly significantly reduced (29.92% and 70.63%) respectively as compared to the control.

The effect of interaction between different doses of gamma-ray and wheat genotypes showed that, 150 Gy of gamma-ray treatment was generally increased plant regeneration in the four wheat genotypes as compared with their controls, Table 3. On the other hand, highly significantly reduction on plant regeneration percentage was obtained with increasing gamma-ray doses to 350 Gy and 450 Gy in the four wheat genotypes as compared to their controls. However, plant regeneration capacity was still more sensitive to gamma radiations than growth as observed in. It could be concluded that low doses of gamma rays could be enhanced plant regernation. From above mentioned results, the four cultivars were divided into-senstive and radio-resistant, whereas, Sids-1 and Sakha-93 genotypes were more resistant to gamma radation than Sahel-1 and Giza-168. Similar results were obtained by Abdrabou and Salam[14] who found that, gamma irradiation at 10 Kr slightly increased percentage of plantlets formation in four wheat genotypes. Also, Rashed *et al*.[4] Abdel-Hady and Abou-Deif[5] in maize and Abdel-Hady and Ahmed[6] in wheat and found that, low dose (150 Gy) of gamma rays has a stimulating effect on agronomic traits of maize and wheat, but high dose caused significant reduction as compared to the control for all studied traits.

4. Correlation among different traits: Estimates of genotypic and phenotypic correlations between callus induction, callus growth rate and grain yield under the four doses of gamma rays are presented in Table 4. The data indicate that the values of the genotypic correlation coefficient (G.C.V.) were comparatively higher than those of the corresponding phenotypic coefficient (P.C.V.). Both types of correlation (G.C.V. and P.C.V.) were in the same direction among studied characters under the four doses of gamma rays. Genotypic and phenotypic correlations were positive and highly significant between callus growth rate and grain yield/plant. This indicated that the strong genetic association between the traits. It be concluded that the tissue culture technique could be valuable for predicting the combining ability. Similar results were obtained by Abd-Hafez and Hamad[15] and Abdel-Hady and Ahmed[6] on maize.

II- Effect of gamma irradiation on some agronomic traits in M_2 generation at harvest:

1. Varietal differences: Data presented in Table 5 indicated that the wheat cultivars: Sids-1, Sakha-93, Sahel-1 and Giza-168 were differed significantly in yield and their components, i.e. plant height, number of tillers per plant, number of spikes per plant, 1000-grain weight (g), grain yield per plant (g) and grain yield per feddan. Moreover, Sid-1 and Sakha-93 significantly exceeded Sahel-1 and Giza-168 in five agronomic traits. Sakha-93 genotype surpassed the three cultivars on plant height.

It could be concluded that varietal differences between wheat cultivars may be due to genetical differences between cultivars, as well as, the range of cultivar response. It is noteworthy to mention that, differences in yield potential of wheat depend undoubtedly on the part of photosynthates partitioned into grain yield. It is worthy to mention that the results of varietal differences in wheat yield and its components were in hormony with the results obtained by Metwally *et al*[16] Hassanein[17] and Ahmed *et al*[18].

2. Effect of gamma rays on yield and its attributes in M_2 generation: Data in Table (5) indicate that treatment of 150 Gy of gamma rays increased significantly in growth characters (plant height and number of tillers per plant) and in yield and its components compared with controls. The average of grain yield (ton/feddan) on 150 Gy gave the highest yield and the percentage of increasing (15.94%) compared with control, while the lowest one at 450 Gy. Application of 250 and 350 Gy caused significantly decreased mean values of all studied characters studied as compared with mean value of control, while 450 Gy of gamma-ray decreased it less than value of control (37.85%). It is clearly noticed that 150 Gy of gamma rays stimulated all character studied. Similar results obtained[3,1].

3. Effect of interaction between wheat cultivars and gamma rays: The interaction between the four wheat

Table 5: Effect of gamma rays on growth, yield and its attributes in M_2 generation of the four wheat genotypes, at harvest.

Wheat genotypes	Gamma doses (Gy)					Genotype Means
	0	150	250	350	450	
Plant height (cm)						
Sids-1	99.1	111.2	94.5	84.4	79.9	93.0
Sakha-93	97.4	107.4	92.6	86.2	81.6	93.8
Sahel-1	94.6	105.3	89.7	79.6	74.8	88.8
Giza-168	93.5	102.9	88.0	78.2	76.0	87.7
Treatment means	96.2	106.7	91.2	82.1	78.1	-
Number of tillers per plant						
Sids-1	9.5	11.6	8.6	6.3	5.1	8.2
Sakha-93	8.2	9.8	6.6	5.7	4.8	7.0
Sahel-1	6.4	7.3	5.7	4.8	3.6	5.6
Giza-168	5.8	7.4	5.3	4.6	3.2	5.3
Treatment means	7.4	8.9	6.5	5.6	4.2	-
Number of spikes per plant						
Sids-1	8.2	11.1	8.3	6.1	5.0	7.7
Sakha-93	6.9	9.4	6.3	5.5	4.6	6.5
Sahel-1	5.7	6.8	5.4	4.6	3.5	5.2
Giza-168	5.0	6.1	5.1	4.3	3.1	4.7
Treatment means	6.5	8.4	6.3	5.1	4.1	-
1000-grains weight (g)						
Sids-1	49.2	54.4	43.9	39.7	36.5	44.7
Sakha-93	46.7	49.8	40.7	37.2	34.3	41.7
Sahel-1	41.3	43.6	39.6	35.1	32.8	38.5
Giza-168	39.1	41.7	37.5	33.4	30.9	36.5
Treatment means	44.1	47.4	40.5	36.4	33.6	-
Grain yield per plant (g)						
Sids-1	13.6	16.8	12.3	10.9	9.1	12.5
Sakha-93	12.2	14.9	11.1	9.3	8.6	11.2
Sahel-1	10.7	12.5	10.8	9.0	7.5	10.1
Giza-168	10.1	11.2	9.3	8.1	6.9	9.1
Treatment means	11.7	13.9	10.9	9.3	8.0	-
Grain yield (ton/feddan)						
Sids-1	2.68	3.15	2.35	2.04	1.68	2.38
Sakha-93	2.64	3.10	2.31	1.98	1.63	2.33
Sahel-1	2.38	2.71	2.28	1.86	1.50	2.15
Giza-168	2.32	2.66	2.19	1.80	1.43	2.08
Treatment means	2.51	2.91	2.28	1.92	1.56	-

Analysis of variance

	L.S.D.	Plant height	No. tillers	No. spikes	1000-grain weight	Grain yield (g)	Grain yield (gon)
Genotype (g)	5%	1.24	0.91	0.94	1.26	0.53	0.11
Gamma (r) doses	5%	1.11	0.82	0.85	1.16	0.46	0.20
GXY	5%	2.48	1.84	1.87	2.22	1.05	0.32

cultivars and gamma ray doses were significant in yield and its components. The interaction between gamma rays at 150 Gy and Sids-1 give a significant increase and surpassed in all growth characters, yield and its component as compared to control, but Sakha-93 give the tallest cultivar at 150 Gy. Giza-168 scorded less the mean value of all characters studied compared with control. The interaction between the four cultivars and 150 Gy gave the highest increase percentage (17.5%, 17.4%, 13.9% and 14.7%) in Sid-1, Sakha-93, Sahel-1 and Giza-168 compared with controls, respectively. Grain yield is a complex trait controlled by many genetic factors. With regard to the four wheat cultivars considerable decreasein the mean of grain yield was noted as dose of gamma rays increased 250, 350, 450 Gy respectively. Increasing yield in irradiated materials over the control could be attributed to the probability that irradiation treatments created a state of heterogenity and activation of some genes that led to increase the yield above unirradiated materials (stimulation effect). Similar results were obtained[4,19].

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