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ORIGINAL ARTICLE

Effect of Gamma Irradiation on Yeild and Yeild Contributing Traits in Hybrids of Sunflower (*Helianthus annuus* L.)

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The seeds of two hybrids FSS64 and SF0049 of sunflower (*Helianthus annuus* L.) were irradiated with different doses of gamma irradiation i.e., 10, 15, 20, 25 Krad from the source of cobalt Co^{60} at Nuclear institute for food and agriculture (NIFA), Peshawar. The irradiated seeds were sown in the research area of the department of Botany, Shaheed Benazir Bhutto University, Sheringal, Dir-Upper during the crop season of 2016. It was noted that a negative correlation was established in doses of 10, 15, 20, 25 Krad versus number of parameters like number of leaves, number of seeds per head and fresh seed weight. With the gradual increase in doses, decreases in mean values for the mentioned parameters were observed. Significant reductions in the mean values were observed for mentioned parameters in both the hybrids FSS64 and SF0049 of sunflower and consistently retardation was recorded with the increasing doses. Direct relationship occurred in the mean values of days taken to germination and maturity while on the other hand means values significantly raised with the rising of doses thus the duration of the mentioned parameters is increased. It was also concluded that rising of doses caused restraining in the mean values of all the parameters, which clarified that both hybrids were sensitive to high doses of gamma irradiation. Furthermore, there were also recovered a few Mutant genotypes in FSS64 hybrid at 15 Krad due to genetic variability with proliferation of two capitula with single peduncle on the main shoot. Differences in the mean square values through analysis of variance with respect to different doses of irradiation for all the parameters were highly significant, while due to the hybrids effect of the number of leaves was non-significant. Moreover, non-significant results were achieved in the mean square values due to the relations between hybrids and doses for days to maturity and the number of leaves.

Key words: Gamma irradiation, Doses, Sunflower hybrid, Mutant, Genotypes, Parameters

Induction of mutations by gamma-rays is widely used in plant breeding programs. Mutation breeding is the most valuable and very important technology of sunflower (Jaya and Selva, 2003) and effectively used in sunflower breeding to change plant productivity and characteristics (Cvejic *et al.,* 2011) through increasing genetic variability (Gvozdenović *et al*., 2009). Mutagens like thermal, fast neutrons, X-rays, beta rays, gamma rays, ultraviolet and infrared radiation have been used (Skoric, 2012) and created numerous mutants with altered, improved vegetative and oil related traits such as dwarf growth, early maturity, thinner husk, oil content and composition etc in sunflower which can be used for cultivation purposes (Cvejic, 2009). The mutation among sunflower inbred lines articulated in the M1 and M2 generations with seven mutants like early flowering, short and high stature, higher oil content and branching along with the stable progeny in the M6 and M7 generations for better seed yield (Cvejic *et al.,* 2015). The higher doses of gamma-rays were noted and observed as lethal and fatal to sunflower crop and therefore, the lower doses were considered as significant and valuable (Giriraj *et al*., 1990). Survival of seedling and fertility of pollen decreased with the increased dose of mutagen in sunflower varieties (Kumar and Ratnam, 2010).

Sunflower (*Helianthus annuus* L., 2n =34) belongs to the family Asteraceae. Sunflower is an affluent source of cooking oil (Friedman *et al*., 2007), contains 40-47% oil and 20-27% protein substance (Saleem *et al*., 2003). It is labeled as fine oil due to the occurrence of 16.2% oleic acid and 72.5% linoleic acid and polyunsaturated fatty acid with 60% (Rathore, 2001). Soluble vitamins A, B, E and K are also included, which is suitable for heart proteins (Gossal *et al.,* 1988), nerves, brain and eyes health. While seeds contained more than 48 calories/tablespoon and additional iron than any other food except for liver and egg yolk (Arshad and Amjad, 2012).

Pakistan is deficient in edible oil production. The demand of edible oil is 77%, which is fulfilled through imports which is 77 %. Edible oil is the second largest import item, which makes Pakistan the third largest importer of edible oils in the world (MINFAL 2006) and cultivated over an area of 877,000 acres and the production is 473,000 tons seed (MINFAL. 2013). Oil seed crops such as sunflower, rapeseed, mustered, canola and cottonseed its share 11.1% of the agriculture sector and 2.3 percent contributes towards Pakistan's gross domestic product (GDP) (Economic Survey of Pakistan, 2014-15). There are a number of advantages of growing sunflower for oil compared with other species. No special machinery is needed to produce this crop. It can be grown as a grasp crop in many situations. Anderson and Olsson (1954) worked out that the increase in yield and oil content in case of Sunflower and white Mustards was due to radiation treatments.

Thus far, Pakistan scientists started to produce sunflower mutants through mutation breeding program to regulate the enhancement of the edible oil production of sunflower to compensate the rising demand of edible oil to save the capital and the economy of the country.

So, the aim of the present study was to investigate the effect of gamma irradiation on some important quantitative traits in sunflower that leads to screen out the effective radiation dose affecting the architecture of irradiated lines in the hilly areas of Sheringal Dir Upper. Moreover, this study is also concern to produce meaningful mutants of sunflower for improving yield of edible oil.

MATERIALS AND METHODS

The experiment is conducted in the crop season of 2016 to conclude the useful dose of gamma radiations on the: FSS64 and SF0049 Hybrids of Sunflowers (*Helianthus annuus* L.) in the study area, Department of Botany, Shaheed Benazir Bhutto University, Sheringal, Dir upper, Khyber Pakhtunkhawa, Pakistan.

The materials (Germplasm) which was utilized in this study is highlighted as follows;

Hybrid 1= FSS64 Hybrid 2= SF0049

The seeds of two Hybrids were purchased from Tarnab Farm, Peshawar and were irradiated with 10, 15, 20, and 25 Krad doses of gamma radiations through Co^{60} gamma source operating at NIFA (Nuclear Institute for Food and Agriculture Peshawar).

The plan of the experimentation was split plot and plot size was kept as 110 x 250 = 27,500 cm^2 areas with three replications and 30 treatments along with control with two Hybrids. The actual experimental plot design was set at 110 x 83.33= 916.67 cm^2 areas with 10 treatments along with control with two hybrids. Seeds were sown in the soil at about 2cm depth. The gap in between two rows was maintained as 75 cm apart while the space between plant-to-plant was kept as 20 cm.

The data of the following parameters were recorded in M_1 generation.

Days taken to Germination.

Data for days to emerge, in each treatment was recorded from the average data of days to emergence of selected plants in that treatment.

Number of Leaves per Plant

Number of leaves per plant was counted by the average value of all the leaves of the chosen plants.

Days taken to Maturity.

Total time acquired for plant maturity was recorded from planting date up to flowering head maturity.

Fresh Seed Weight per Head (mg).

Fresh seed weight per head was noted in grams.

Number of Seeds per Head.

Total number of seeds per head was taken from the average value of the number of seeds per head of the selected plants.

Statistical analysis:

Recorded data were analyzed using statistic software Statistix 8.1. Tukey's Honestly Significant Difference Test was applied for comparison among the treatments means, hybrids means and the interactions of treatments under hybrids.

RESULTS AND DISCUSSION

1. Days before Germination

The analysis of variance according to the table 1a depicted that the effect of hybrids into radiation dose interaction was statistically significant and the effect of radiation doses and effect of hybrids was statistically highly significant ($P < 0.05$).

The data according to the table 1b and figure 1 exhibited that the hybrid SF0049 took more days to germination (13 days), whereas the hybrid FSS64 germinated earlier (10.42 days). The duration of germination was also analyzed as statistically significant in the two hybrids under study.

The radiation also differed in the days taken to germinate. The untreated took the least number of days (8.20) which was, however, non-significant statistically comparatively to10 Krad (9.00 days). The highest dose that is 25 Krad took the maximum days to germinate (16 days).

A considerable variation was noted in all the interaction of hybrids against different doses. The interaction of FSS64 hybrid into 10 Krad took the least number of days (7.33 days), however, it was statistically non-significant from the same hybrid in comparison under check (7.39 days). The hybrid FSS64 with 25 Krad took maximum days (15.34 days). The interaction of control and hybrid SF0049 took the least number of days (9.00days) which was statistically non-significant from the same hybrid relatively to 10 Krad (10.67 days). The interaction of hybrid SF0049 with 25 Krad took the highest days to germination (16.67days). The means of hybrid SF0049 with different dose interactions was not significantly different from one another. However, the interaction of hybrids and radiation doses was statistically significant.

It is concluded that, hybrid FSS64 under control took the minimum number of days and hybrid SF0049 radiated with 25 Krad took the upper limit of days.

2. Number of Leaves per Plant

The analysis of variance according to Annexure table-2a, represented that the effect of hybrids and the hybrids under different radiation doses were statistically non-significant ($P > 0.05$), whilst the effect of radiation doses was highly significant statistically ($P <0.05$).

The data according to the table 2b and figure 2 showed that the difference in parameter like number of leaves per plant produced by FSS64 hybrid (25.84) and SF009 hybrid (24.44) was statistically noted as non-significant.

The result of radiation doses also changed the number of leaves per hybrid. The untreated produced more number of leaves (31.74) as compared to radiated hybrids. Least number of leaves (18.37) were produced at 25 Krad, which was highest dose. However, the difference in the number of leaves among the radiation doses was statistically significant.

The interaction between hybrids and different doses of irradiation showed that the interaction of untreated FSS64 hybrid produced more number of leaves (32.93) whereas the less number of leaves were produced by the same hybrid under 25 Krad (19.1). However, untreated hybrid SF0049 produced more number of leaves (30.54) and retardation was noted in the same hybrid under 25 Krad and produced the less number of leaves (17.66). Moreover, the Hybrid FSS64 as untreated produced more number of leaves (32.93) as compared to control hybrid SF0049 (30.54). The difference was statistically non-significant among the interactions of different doses verses both hybrids.

3. Days taken to Maturity

The analysis of variance in table 3a, demonstrated that the effect of radiation doses was highly significant and hybrids were statistically significant ($P < 0.05$). Along with, the effect of interaction of radiation doses verses hybrids were statistically non-significant ($P > 0.05$).

Table 3b and figure 3 represents that the hybrid SF0049 acquired more days to maturity (123.93) while hybrid FSS64 kept utilize less days to maturity (115.47 days). The difference in period of maturity was statistically significant in the both hybrids.

Furthermore, the untreated hybrid FSS64 took the minimum number of days (113 days) while the same hybrid with 25 Krad took the maximum number of days (118.67 days). In addition to the controlled hybrid SF0049 which took the minimum number of days (121 days) as well as maximum number of days of the same hybrid with 25 Krad acquired (126.67 days). Hybrid FSS64 matured prior than SF0049 in comparison with control and other with different doses. Even though the differences in the interaction of doses verses hybrids were also noted statistically non-significant.

The radiation doses also varied in days taken to maturity. The control took the minimum number of days (117 days), however, it was statistically non-significant of the same hybrid under 10 Krad (118.17). Although, the highest dose that is 25 Krad took the maximum number of days (122.67 days) to mature. So far, the difference between the radiation doses was statistically significant.

4. Fresh Seed Weight per Head

The analysis of variance of table- 4a, illustrated that the effect of interaction of hybrids and doses along with the effect of hybrids were statistically significant, however the effect of radiation doses was statistically highly significant ($P < 0.05$).

According to table-4b figure-4 it is shown that the hybrids FSS64 achieved higher fresh seed weight / head (78.80 gm), while hybrid SF0049 achieved the lesser fresh seed weight (76.69 gm). The difference in both the hybrids was statistically significant.

The difference among different doses in response to gamma irradiation was statistically significant. The highest fresh seed weight/head were recorded in untreated (103.63 gm) and lowest fresh seed weight (61.45 gm) was achieved with higher dose (25 Krad).

Similarly, the interaction of different doses with hybrids also showed significant differences among the different interactions. However, the hybrid FSS64 under control gained the highest fresh seed weight/head (104.60 gm), while less fresh seed weight gained by hybrid FSS64 with 25 Krad (62.60 gm). Hybrid SF0049 under control gained the highest fresh seed weight /head (100.67gm) and lesser fresh seed weight/head gained by SF0049 hybrid with 25 Krad (60. 30 gm) comparatively. In the same way Hybrid FSS64 gained the highest fresh seed weight (104.60 gm) in comparison to hybrid SF0049 (100.67 gm) whereas the hybrid SF0049 gained the lesser fresh seed weight per head (60.30 gm to hybrid FSS64 (62.60 mg).

5. Number of Seeds per Head

As far as the analysis of variance according to the table 5a, the effect of hybrids, hybrids verses doses and the effect of different doses in response of gamma radiation was observed as statistically highly significant ($P < 0.05$).

Table 5b and figure 5 revealed that hybrid FSS64 produced more number of seeds per head (775. 60) in comparison to hybrid SF0049 which produced less number of seeds (656.53). The difference between both the hybrids was statistically significant. Nevertheless, the yield of FSS64 hybrid was recorded more than SF0049.

The radiation doses also produced a variable number of seeds per head. The different doses of radiation fluctuated variably but more number of seeds (1085.2) were recorded in case of control, although the less number of seeds (454.8) was achieved under highest dose (25 Krad). Similarly, the difference between the radiation doses was significant statistically.

The interaction of control in relation to hybrid FSS64 produced more number of seeds (1195.7) and the same hybrid under radiation dose of 25 Krad produced a less number of seeds (461.3). Also the hybrid SF0049 under control produced more number of seeds per head (974.7) and the same hybrid with 25 Krad produced a less number of seeds (448.3). The hybrid SF0049 under 20 Krad exposures in comparison to the same hybrid under 15 Krad, the difference was however noted as non-significant statistically. Moreover, hybrid FSS64 under control produced more number of seeds per head (1195.7) as compared to control with hybrid SF0049 (974.7). In the same way less number of seeds per head produced by SF0049 under 25 Krad (448.3) in comparison to the hybrid FSS64 under 25 Krad (461.3).

So far as the comparison with the previous researchers is concerned, the same results for Significant reduction for days taken to germination due to radiation doses is also revealed by Majeed *et al*., (2009) on *Lepidum sativum* L. Din *et al.,* (2003) in case of wheat genotypes is also observed significant mean square values of radiation doses, varieties and varieties under interaction of doses for earing initiation and earing initiation increased as radiation doses and varieties under interaction of doses increased excluding 15 Krad, which decreased the interval of germination.

While it seem dissimilar conclusive as reported by Borzouei *et al.,* (2010) in case of wheat (*Triticum aestivum* L.) with increasing dose resulting in decreasing the germination time. This fluctuation of the data may depict the research trials under different environmental factors on different genotypes.

Table-1a: Analysis of variance for days taken to germination in sunflower hybrids as affected by gamma rays.

Source	**DF**	**SS**	**MS**	**F**	**P**	**Sig**
Replications	2	2.662	1.3311			
Hybrids	1	48.490	48.4901	50.37	0.0193	**
Error Rep*Hybrids	2	1.925	0.9627			
Treatment	4	265.074	66.2685	138.90	0.0000	**
Hybrids*Treatments	4	6.141	1.5352	3.22	0.0406	*
Error Rep*Hybrids*Trt	16	7.633	0.4771			
Total	29					

Abbreviations in tables representing: DF (degree of freedom), SS (sum of square), MS (means square), F (fisher), P (probility value), Sig (significant via Tukey HSD test), ** (highly significant), * (significant)

Table-1b: Effect of gamma irradiation on days taken to germination in Sunflower hybrids.

TREATMENTS	**FSS64**	**SF0049**	**MEAN**
T0 (CONTROL)	7.389^{D}	9.000^{C}	8.194 D
T1 (10 KRAD)	7.333^{D}	10.667^{C}	9.000 D
T2 (15 KRAD)	9.722^{C}	13.333^{AB}	11.528C
T3 (20 KRAD)	12.333^{B}	15.333^{A}	13.833B
T4 (25 KRAD)	15.333^{A}	16.667^{A}	16 A
MEAN	10.422B	13.422 A	

Any two means sharing same letters are not significantly different from one another according to the Tukey HSD test used for all pair wise comparison. Capital letters indicate significance at the 5% probability level.

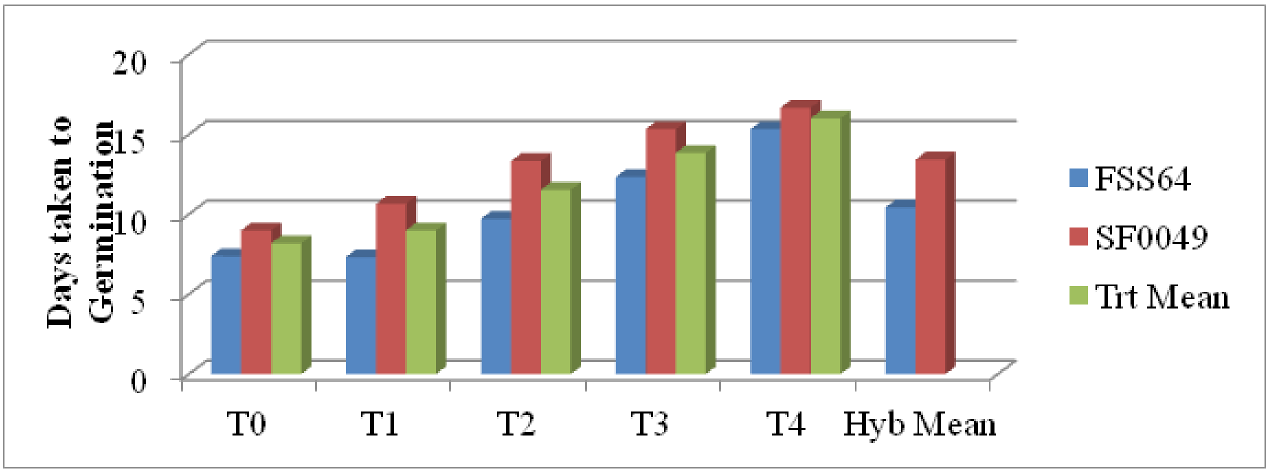

Figure-1. Effect of gamma irradiation on days taken to germination in Sunflower hybrids

Table-2a. Analysis of variance for the number of leaves per plant in sunflower hybrids as affected by gamma rays.

Source	DF	SS	MS	F	P	Sig
Replications	2	10.968	5.484			
Hybrids	1	19.200	19.200	1.93	0.2992	NS
Error Rep*Hybrids	2	19.896	9.948			
Treatment	4	645.939	161.485	69.48	0.0000	**
Hybrids*Treatments	4	5.320	1.330	0.57	0.6867	NS
Error Rep*Hybrids*Trt	16	37.189	2.324			
Total	29	738.512				

Abbreviations in tables representing: DF (degree of freedom), SS (sum of square), MS (means square), F (fisher), P (probility value), Sig (significant via Tukey HSD test), ** (highly significant), * (significant)

Table-2b. Effect of gamma irradiation on number of leaves per plant in Sunflower hybrids.

TREATMENTS	FSS64	SF0049	MEAN
T0 (CONTROL)	32.933^{A}	30.53^{A}	31.733A
T1 (10 KRAD)	29.733^{AB}	27.0^{AB}	28.367B
T2 (15 KRAD)	25.667^{BC}	25.13^{BC}	25.4C
T3 (20 KRAD)	22.800^{CD}	21.8^{CD}	22.333D
T4 (25 KRAD)	19.067^{D}	17.66^{D}	18.367E
MEAN	25.840A	24.440A	

Any two means sharing same letters are not significantly different from one another according to the Tukey HSD test used for all pair wise comparison. Capital letters indicate significance at the 5% probability level.

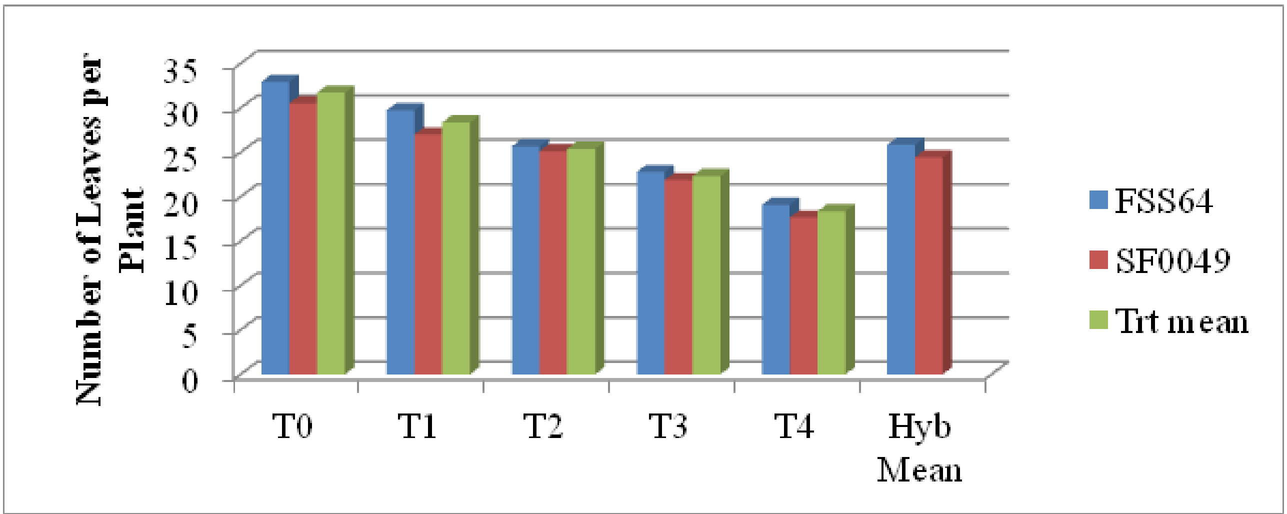

Figure-2. Effect of gamma irradiation on number of leaves per plant in Sunflower hybrids.

Table-3a. Analysis of variance for days taken to maturity in Sunflower hybrids as affected by gamma rays.

Source	DF	SS	MS	F	P	Sig
Replications	2	29.600	14.800			
Hybrids	1	537.633	537.633	93.77	0.0105	**
Error Rep*Hybrids	2	11.467	5.733			
Treatment	4	123.800	30.950	48.23	0.0000	**
Hybrids*Treatments	4	1.533	0.383	0.60	0.6698	NS
Error Rep*Hybrids*Trt	16	10.267	0.642			
Total	29	714.300				

Abbreviations in tables representing: DF (degree of freedom), SS (sum of square), MS (means square), F (fisher), P (probility value), Sig (significant via Tukey HSD test), ** (highly significant), * (significant)

Table-3b. Effect of gamma irradiation on days taken to maturity in sunflower hybrids.

TREATMENTS	FSS64	SF0049	MEAN
T0 (CONTROL)	113.000^{C}	121.00^{D}	117.000D
T1 (10 KRAD)	113.67^{C}	122.33^{CD}	118.17CD
T2 (15 KRAD)	115.33^{BC}	123.67^{BC}	119.50C
T3 (20 KRAD)	116.67^{AB}	125.67^{AB}	121.17B
T4 (25 KRAD)	118.67^{A}	126.67^{A}	122.67A
MEAN	115.47B	123.93A	

Any two means sharing same letters are not significantly different from one another according to the Tukey HSD test used for all pair wise comparison. Capital letters indicate significance at the 5% probability level.

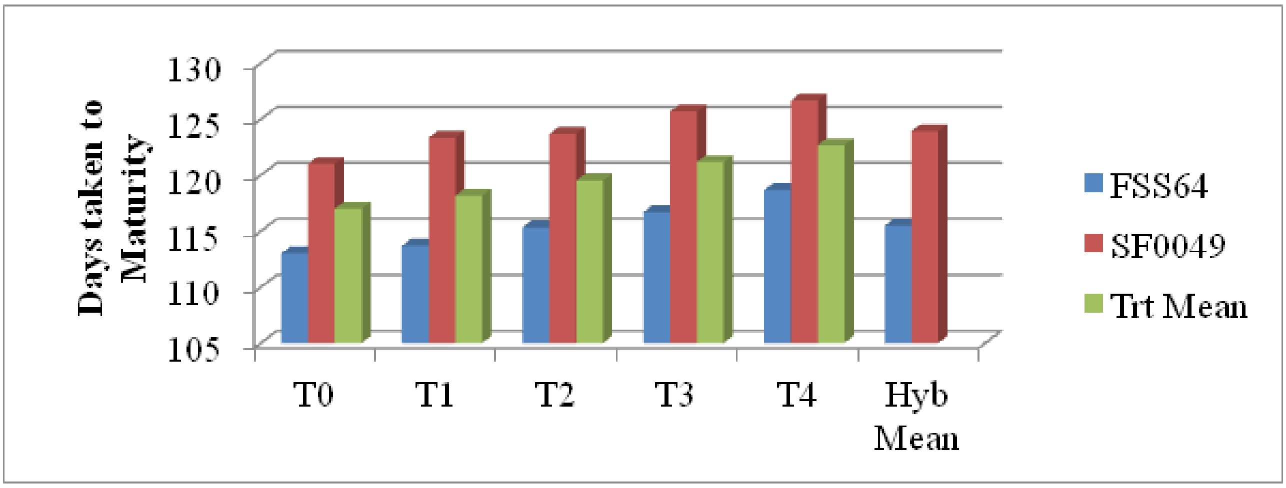

Figure-3. Effect of gamma irradiation on days taken to maturity in sunflower hybrids.

Table-4a. Analysis of variance for fresh seed weight in (gm) per head in Sunflower hybrids as affected by gamma rays.

Source	DF	SS	MS	F	P	Sig
Replications	2	0.27	0.13			
Hybrids	1	33.50	33.50	438.82	0.0023	**
Error Rep*Hybrids	2	0.15	0.08			
Treatment	4	6959.29	1739.82	39097.1	0.0000	**
Hybrids*Treatments	4	9.38	2.3	52.67	0.0000	**
Error Rep*Hybrids*Trt	16	0.71	0.04			
Total	29	7003.29				

Abbreviations in tables representing: DF (degree of freedom), SS (sum of square), MS (means square), F (fisher), P (probility value), Sig (significant via Tukey HSD test), ** (highly significant), * (significant)

Table-4b. Effect of gamma irradiation on fresh seed weight in (gm) per head in sunflower hybrids.

TREATMENTS	FSS64	SF0049	MEAN
T0(CONTROL)	104.60^{A}	100.67^{A}	102.63A
T1(10 KRAD)	87.93^{B}	86.50^{B}	87.22B
T2(15 KRAD)	72.30^{C}	71.73^{C}	72.02C
T3(20 KRAD)	66.57^{D}	64.23^{D}	65.40D
T4(25 KRAD)	2.60^{E}	60.30^{E}	61.45E
MEAN	78.8A	76.69B	

Any two means sharing same letters are not significantly different from one another according to the Tukey HSD test used for all pair wise comparison. Capital letters indicate significance at the 5% probability level.

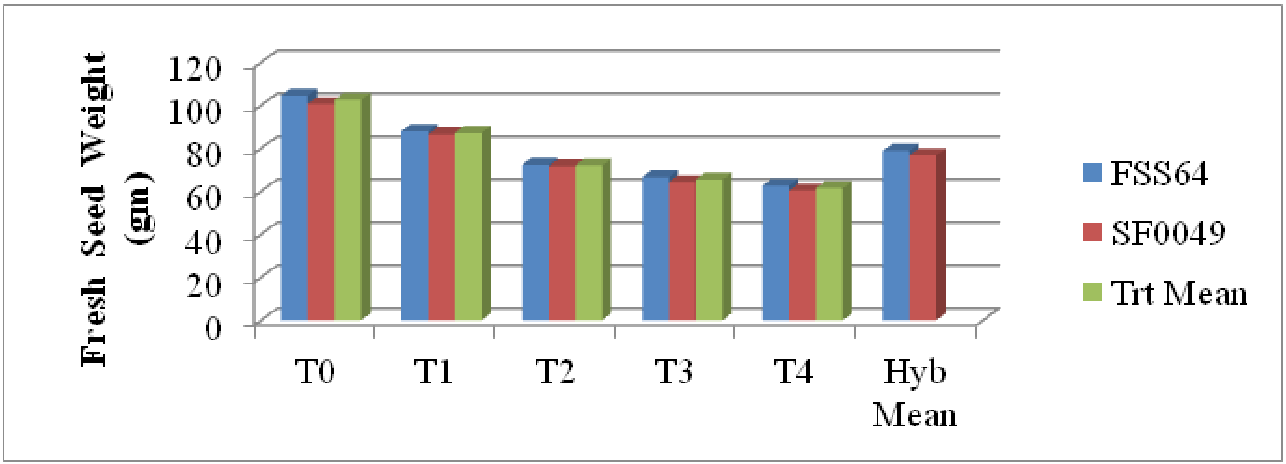

Figure-4. Effect of gamma irradiation on fresh seed weight in (gm) per head in sunflower hybrids.

Table-5a. Analysis of variance for the number of seeds per head in sunflower hybrids as affected by gamma rays.

Source	DF	SS	MS	F	P	Sig
Replications	2	371	186			
Hybrids	1	106327	106327	5007.53	0.0002	**
Error Rep*Hybrids	2	42	21			
Treatment	4	1710553	427638	949.04	0.0000	**
Hybrids*Treatments	4	54147	13537	30.04	0.0000	**
Error Rep*Hybrids*Trt	16	7210	451			
Total	29	1878650				

Abbreviations in tables representing: DF (degree of freedom), SS (sum of square), MS (means square), F (fisher), P (probility value), Sig (significant via Tukey HSD test), ** (highly significant), * (significant)

Table-5b. Effect of gamma irradiation on number of seeds per head in sunflower hybrids.

TREATMENTS	FSS64	SF0049	MEAN
T0(CONTROL)	1195.7^{A}	974.7^{A}	1085.2A
T1(10 KRAD)	1002.0^{B}	796.0^{B}	899.0B
T2(15 KRAD)	679.7^{C}	560.3^{C}	620.0C
T3(20 KRAD)	539.3^{D}	503.3^{C}	521.3D
T4(25 KRAD)	461.3^{E}	448.3^{D}	454.8E
MEAN	775.60A	656.53B	

Any two means sharing same letters are not significantly different from one another according to the Tukey HSD test used for all pair wise comparison. Capital letters indicate significance at the 5% probability level.

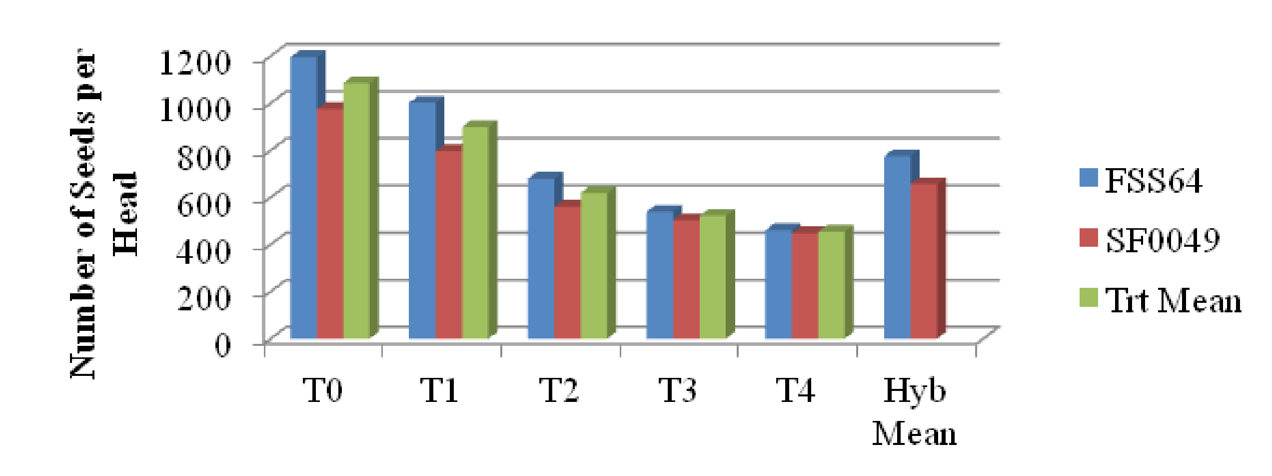

Figure-5. Effect of gamma irradiation on number of seeds per head in sunflower hybrids.

The number of leaves significantly decreased as radiation doses increased, causing a diminution in the number of leaves per plant. This result is also confirmed by Majeed *et al.,* (2009) in *Lepidium Sativum* l., Gunasekaran and Pavadai, 2015 at *Arachis hypogaea* L., and El-Khateeb *et al*., 2016 at *Philodendron scandens* plant*.* The similarity in results might be attributed due to similar genetic response to the environment instead of different locations where the experiments were carried out.

Din *et al.,* (2003) have also confirmed significant difference in mean square of the hybrids at different doses and the effect of interaction of hybrids radiated with different doses for earing completion. Nevertheless, our results are analogous to the significant mean square value due to the effect of hybrids and the effect of different doses, but deviate based on the effect of interaction of hybrids and different doses. Particularly days taken to maturity significantly increased as radiation doses increased caused retardation in the days taken to maturity. The result is also confirmed by Sharif *et al., (*2000) on *Gossypium hirsutum* L, Majeed *et al*., (2009) in *Lepidium Sativum* L., and Din *et al.,* (2003) in *Triticum aestivum* L.

Jagadeesan (2008) is also revealed similar result of fresh seed weight except the mean value at 15 Krad which was highest and then decreased due to different radiation dose.

Significant decreasing in the number of seeds per head with the increasing dose of gamma irradiation results were similar as recorded by Khah and Verma (2015) on wheat (*Triticum aestivum* L.) var. WH-147 and Akgun and Metin (2004) on perennial rye (*Secale montanum* Guss.), Pushparajan (2014) on Okra except 400 Gy but quite similar to Jagadeesan *et al.,* (2008) on sunflower but dissimilar to the mean value at 10 Krad which was highest among all the doses and then gradual decreased at 15, 20 and 25 Krad.

CONCLUSUSION

A reduction was obtained in most parameters with the increasing doses of gamma irradiation because of the injurious effect of exceeding the dose of radiation supplemented by agroclimatic and environmental factors such as soil texture, soil chemistry, temperature, humidity, light intensity and especially due to high altitude. Both hybrids were estimated as sensitive to the high doses of gamma rays leading to a reduction in the mean values of the most of the parameters. The 15 Krad dose had the ability to induce mutation in FSS64 hybrid and became the source of change in the genetic make-up in the shape of a better result in yield and yield components.

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