J. Appl. Res Plant Sci. Vol. 6(1), 08-17, **2025,** https://doi.org/10.38211/joarps.2025.06.300

Research Article

OPEN ACCESS

Available on https://www.joarps.org
Journal of Applied Research in Plant Sciences
(JOARPS)
ISSN: 2708-3004 (Online), 2708-2997 (Print)

Mutagen-induced Genetic Variability and Heritability Analysis for Yield Associated Quantitative Characters in Canola

Zeenat Bano[1], Sadaf Tabasum Qureshi[1*], Rabia Asma Memon[1], Imtiaz Ahmed[2]

[1]Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan,
[2]Nuclear Institute of Agriculture, Tando Jam
Corresponding authors Email: sadaf.qureshi@usindh.edu.pk

Article Received 12-08-2024, Article Revised 03-12-2024, Article Accepted 14-02-2025

ABSTRACT

Due to adverse effect of other cooking oils, people are switched on health promoting oils and vegetables; therefore canola genetic improvement regarding yield has been planned in majority of European and Asian countries. The major advantage of canola green and oil is for availability unsaturated fatty acid which indirectly improves blood pressure by thinning blood (dissolve many nutrients, vitamins and cholesterol) cardiac activities and muscles strength. Genetic variability through mutagenesis is proven as a powerful tool for introducing new alleles, therefore different doses of Gamma radiations (750Gy, 1000Gy, 1250Gy) and Ethyl methansalfonate (0.5%, 1.0% and 1.5%) were used for grain yield associated quantitative characters in two Canola genotypes (Hyola-42 and Shiralee). The data was recorded for number of seeds per pod, number of pods per plant, 1000 seed weight and seed yield per plant in M2 and M3 generation. All mutagenic treatments had a significant ($p \leq 0.05$) variation on all traits studied and produced great variability for both mutagens. The mean value of all the phenotypic parameters in M2 generation increased with moderate dose of EMS and Gamma rays in both genotypes as compared to respective controls and in M3 generation both genotypes were more responsive to Gamma rays (1000Gy) for all studied characters in both tested genotypes. Heritability analysis exhibited high heritability and high genetic advance for most of the traits by moderate and highest does of Gamma rays, while reverse is true for EMS in both generations for Shiralee. Over all gamma rays was most efficient to induce better allele contributing to 1000-seed weight.

KEYWORDS: Mutagen, yield, quantitative characters, Canola, Germ plasm

INTRODUCTION

Due to adverse effect of other cooking oils, people are switched on health promoting oils and vegetables; therefore canola genetic improvement regarding yield has been planned in majority of European and Asian countries. The major advantage of canola green and oil is for availability of omega-6, omega-3, vitamin K2 and Lenolenic acid. All these constituents directly or indirectly improves blood pressure by thinning blood (dissolve many nutrients, vitamins and cholesrtol) cardiac activities and muscles strength (Sharafi *et al.*, 2015; Braatz *et al.*, 2017; and Panda *et al.*, 2024).

Rapeseed (*Brassica napus* L) is the second most important oil crop after cotton and sunflower in Pakistan. It is an important oilseed crop, good source of vegetable oil as well as protein-rich meal. Rapeseed oil is mainly used in human nutrition and biofuel production. (Okuzai *et al.*, 2018; Gupta *et al.*, 2024).

In Pakistan the shortage of edible oil is increasing rapidly and the country has faced the adverse of economic threat by the deficiency of edible oil (Aslam *et al.*, 2000). Substantial amount of foreign exchange is spent each year on the import of health promoting edible oils in Pakistan. Demand for edible oils is increasing with an increase in population and health consciousness amongst the people. Oleiferous brassica occupy an important position in the oilseed crops in our country but its production per unit area is lower than other countries in the world (STATIST, 2021).

Crops with narrow genetic base like rapeseed (Anwar *et al.*, 2015; Siddiqui *et al.*, 2021), the use of mutagenesis is the best option to induce novel genetic variability (Chaudhary *et al.*, 20 19). In recent years, both gamma rays and Ethyle methansulfonate were mainly used to ti increase seed yield (Ali and Shah, 2013; Emrani *et al.*, 2015; Hussain *et al.*, 2017; Lee *et al.*, 2018; Gupta *et al.*, 2022; Panda *et al.*, 2024). Therefore, main objective of current study were to evaluate and compare M_2 and M3 mutants of Shiralee and Hyola-42, for four important yield associated parameters.

MATERIAL METHOD

Present work is planned to provide the comparative effectiveness of physical and chemical mutagens on Canola (*Brassica napus* L.) genotypes Hyola-42 and Shiralee for improving some yield associated quantity characters. The plants with improved characters will be used in future by plant breeders as parent in other crosses for variety development. The induced improvement in yield associated characters will be helpful in reducing malnutrition and canola essential oil. The increase in quality oil production will help in reducing the shortage of healthier edible oil and its price for common people.

Experimental field: Current study was conducted at Nuclear Institute of Agriculture (NIA), Tando Jam, Pakistan, during cropping season 2018-2019 and 2019-2020. It is located at an elevation of None meters (0 feet) above sea level, Tando Jam has a Subtropical desert climate. The district's yearly temperature is 33.61ºC (92.5ºF) and it is 12.72% higher than Pakistan's averages. Tando Jam typically receives about 16.51 millimeters (0.65 inches) of precipitation and has 20.65 rainy days (5.66% of the time) annually (https://weatherandclimate.com).

Experimental plant material: Two best hybrid verities of canola introduced from abroad Hyola-42 and Shiralee were selected for further genetic improvement. The seeds of these varieties were obtained from Nuclear Institute of Agriculture (NIA) Tando Jam Sindh.

Mutagen employed: The chemical mutagen, Ethyl Methane Sulphonate (EMS) (0.5 %, 1.0 % and 1.5 % and physical mutagen, Gamma rays (750 Gy, 1000 Gy and 1250 Gy) were applied, to improve early yields associated Quantitative parameters of Canola.

EMS treatments were carried out in Plant Tissue Culture and Biotechnology Research Laboratory of NIA Tando Jam and Gama rays treatments were done at Nuclear Institute of medicine and radiotherapy (NIMRA) Jamshoro. To compare the genetic improvement by each mutagen untreated seeds were used as control. The control and gamma rays treated seeds were soaked in distilled water for 10 hours while EMS treatment of 4h was given to 6h presoaked seeds in distill water (total 10hrs).

Experimental Design: To carry out all experiments Randomized complete Block Design (RCBD) with three replications was used. The plot size was 4 x 3 m = 12 m^{-2}.

Raising of M1, M2 and M3 generations: Mutagen treated seeds (M0) of both varieties were sown in 4m long plots. The row distance was maintained at 30 cm and plant to plant distance was 20cm while raising M1, M2 and M3 generations. After every 20 rows of mutated seeds two rows of respective control verities were planted to make comparison process easier for selection. For sowing each generation five pods from each plant were harvested and bulked dose-wise for each treatment. Selections for the desired traits were carried out on twenty plants from each generation (M2 and M3) at random per treatment. The five randomly selected plants per treatment were used in each generation.

Parameter studies: The data recording was done by the following methods, Simple counting was done to record number of seed per pod, number of pod per plant but 1000 seed weight and yield per plant were done by digital weight machine.

Statistical analysis: Data of quantitative characters of M2 and M3 was statistically analyzed through Statistica version 8.1. Initially two-way Analysis of Variance (ANOVA) was done and further means compared by Least Significant Difference test at $p \leq 0.05$ (31).

Heritability analysis: The four important yield associated quantitative traits of M2 and M3 generation were further subjected to heritability analysis and compared with respective (parents) untreated control. Genetic parameters were calculated as suggested by (Falconer *et al.*, 1977, & Singh *et al.*, 1985) with a selection intensity of 5% according to (Larik *et al.*, 1997).

1. Mean x 2 = ∑xi
2. Variance S 2 = SS
n-1
3. Standard deviation (SD) =
4. Coefficient of variability (CV) = X 100
5. Genetic variance (Vg) = VF 3 -Ve
6. Environmental variance (Ve) = (VP 1 +VP 2) / 2
7. Heritability in broad sense (H 2 b.s%) = Vg/ VF 3 × 100
8. Genetic advance (G.A) = K× (H) × SD

All the means of genetic parameters were computed by least significant difference (LSD)
and standard error (SED) sing the following formula:
LSD = SED X t (0.05) df = degree of freedom.
n = for number of replications where EMS = error mean square
T (0.05) df = t distributes on value form the table at probability 5% level and error degree of freedom.

RESULT & DISCUSSION

In this study two mutagens Gamma rays and EMS were applied on Canola genotypes: Hyola-42 and Shiralee for the analysis of four yield associated quantitative characters. Evaluation of analysis of variances and mean comparison were achieved by DMRT (Duncan's Multiple Range Test). While compiling the results it was not relied on mean comparison but also evaluate up to Heritability measurements for selection of a particular character.

The Result of analysis of variance (ANOVA) of quantitative characters

Analysis of variance (ANOVA) using Duncan's Multiple Range Test (DMRT): The results of ANOVA (Table 1 & 2) for Quantitative characters i.e., number of seeds /pod and number of pods /plant showed that varieties, treatments and varieties × Treatment were not significantly different ($P \leq 0.05$) in

both M2 and M3 generation but 1000 seed weight and seed yield/plant revealed that treatment were non-significant (P≤ 0.05) and varieties and varieties × treatment are significantly different in M2 generation and M3 generation of both the tested genotypes Hyola-42 and Shiralee.

Table 1. Mean square (MS) and ANOVA performance of different Quantitative traits in two canola varieties (Hyola-42 and Shiralee) of M2 generation**.**

S. No	Parameters	Replication	Varieties	Treatments	V x T	Error	Grand mean
	D. F	2	1	6	6	26	
1	Number of seed/Pod	0.0064	1.2002	32.6515	2.0402	0.6616	17.521
2	Number of Pod/Plant	50.7	1620.9	34229.0	1387.0	152.3	278.26
3	1000 seed weight	0.00288	0.00009	3.33123	0.16188	0.00092	2.3000
4	Seed yield/Plant	0.0063	0.1610	32.3257	0.9784	0.0024	8.4519

(NS/P = number of seed/pod, P/P = number of pod/plant, 1000S.W = 1000 seed weight, S.Y/P = seed yield/plant)

Table 2. Mean square (MS) and ANOVA performance of different Quantitative traits in two canola varieties (Hyola-42 and Shiralee) of M3 generation

S. No	Parameters	Replication	Varieties	Treatments	V x T	Error	Grand mean
	D. F	2	1	6	6	26	
1	Number of seed/Pod	0.2124	22.7336	62.2004	17.6413	0.0975	17.545
2	Number of Pod/Plant	80.3	8400.9	46076.4	3495.4	63.8	287.68
3	1000 seed weight	0.00287	0.04667	6.53494	0.26698	0.00546	2.5690
4	Seed yield/Plant	0.0089	0.1539	59.9292	0.6140	0.0103	8.7895

(NS/P = number of seed/pod, P/P = number of pod/plant, 1000S.W = 1000 seed weight, S.Y/P = seed yield/plant)

Mean comparison for number of seeds per pod: The data for number of seeds per pod exhibited in the (Figure.1). In M2 generation gamma rays showed highest value (20.400) at 1000 Gy in Hyola-42 while EMS gave highest mean value at 1.0% (19.867) higher than control (17.300) while Shiralee has highest mean value at 1000 Gy (19.500) irradiated by Gamma rays where as EMS gave highest value at 1.0% (18.533) higher than control (18.300). In M3 generation Gamma rays generated highest value at 1000 Gy (22.067) in Hyola-42 while EMS gave highest value (20.300) at 1.0% EMS higher than control (18.367) but Shiralee has highest mean value at 1000 Gy (21.167) irradiated by Gamma rays where as EMS gave highest value at 1.0 % (19.800) higher than control (19.100). Among all the applied doses of EMS and Gamma rays, 1000 Gy of gamma rays gave highest mean value (19.950) for number of seeds per pod for both the tested genotypes in M2 and the 1000 Gy gave highest mean value (21.617) in M3 generation. Hyola-42 has highest grand mean value (17.690) than Shiralee (17.352) in M2 generation while Hyola-42 has highest grand mean value (18.281) for number of seeds per pod than Shiralee (16.810) in M3 generation.

The analysis of variance regarding number of seeds per pod (Figure.1) indicated that among all the treatments of Gamma rays and EMS, the moderate dose of gamma rays induced maximum number of seeds per pod than control in both genotypes. The moderate dose of Gamma rays was best for both the tested genotype (Hyola-42 and Shiralee). Among the genotypes, Hyola-42 responded best to all the treatments of Gamma rays and EMS; it induced maximum number of seed per pod. In Hyola-42 all mutagenic treatments increased the values for this trait than control. The closest results also observed in the work of different researcher in the world. The lower and moderate doses of gamma rays induced maximum numbers of seed per pod in different Brassica varieties (Shah *et al.*, 1990; Siddiqui *et al.*, 2009; Khan *et al.*, 2014). The medium dose increased the number of seed per pod in M2 and M3 lines in canola (Emrani *et al.*, 2012).

In M3 generation results regarding the number of seeds per pod (Figure.1) indicated that lower and medium dose of Gamma radiations induce maximum number of seeds per pod as compared to high level dose and control. Towards all treatments of Gamma rays and EMS, Hyola-42 showed an effective response for this character.

Other researchers reported nearly similar effect of moderate doses of Gamma rays on pods per plant in canola (siddiqui *et al.*, 2009; Malod *et al.*, 2016; Amosava *et al.*, 2019; Addai *et al.*, 2019).

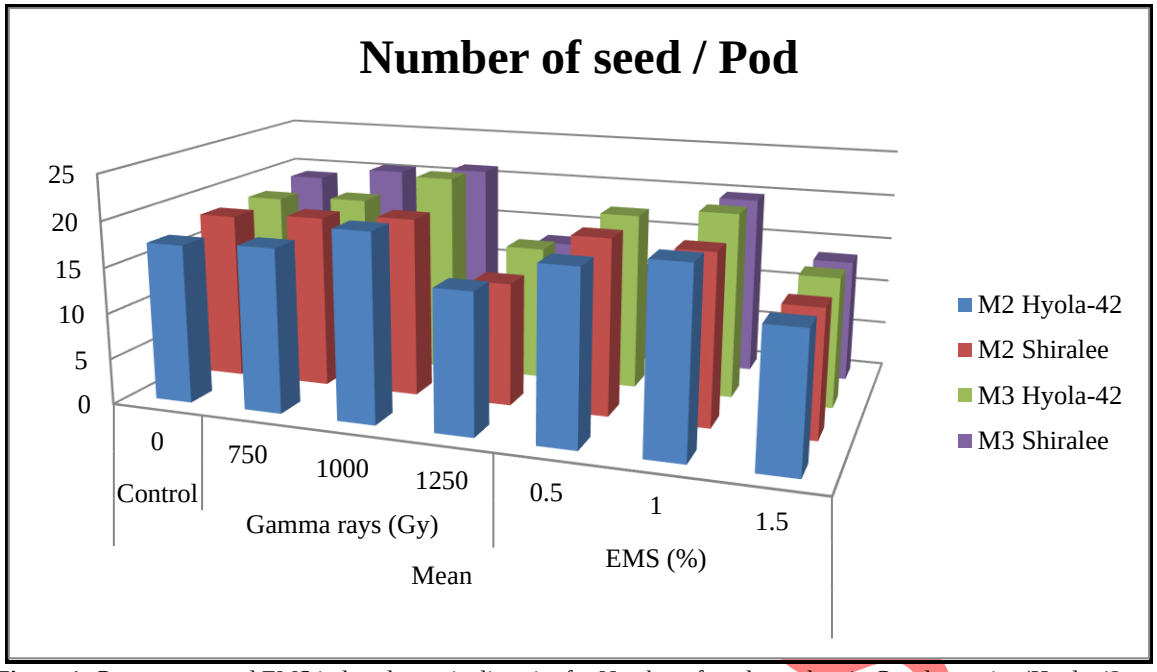

Figure 1. Gamma rays and EMS induced genetic diversity for Number of seed per plant in Canola species (Hyola-42 and Shiralee) in M2 and M3 generation.

Heritability analysis for Number of seeds per pod: For the character of Number of seeds per pod 1000 Gy of gamma rays produced high heritability (h^2=40.76%) with low genetic advance (GA=0.42) in M2 generation and lowest heritability (h^2=1.73%) observed at 1000 Gy in M3 generation in Hyola-42. In case of Shiralee high heritability in broad sense (h^2=70.00%) along with low genetic advance (GA=0.28) produced by 1250 Gy in M2 generation and moderate heritability (h^2=50.00%) with lowest genetic advance (GA=0.13) observed at all the applied doses of gamma rays and EMS except 1000 Gy in M3 generation for number of seed per pod (Table.3).

The evaluation of an important character the number of seeds per pod (Table.3) revealed that in M2 generation moderate heritability and low genetic advance observed in both genotypes Hyola-42 and Shiralee. Whereas moderate heritability and very low genetic advance showed by Shiralee in M3 generation but in case of Hyola-42 very low heritability and least genetic advance observed in M3 generation. Gamma rays did not increase the heritability values in M3 generation but the high values of Heritability were observed with 1000 and 1250 Gy in M2. Current results are consistent with the work of many researchers who find high heritability and genetic advance for polygenes prevailing characters when subjected to mutagenesis (Shah *et al.*,1990; Zeng and Liu, 2022; Panda *et al.*, 2024).

Table 3. Genetic parameters of M2 and M3 Canola genotypes (Hyola-42 and Shiralee) affected by Gamma rays and EMS for number of Seed / Pod.

Treatments	M2 Mean	v.p	v.g	h^2	G.A	M3 Mean	v.p	v.g	h^2	G.A
Hyola-42										
Control	17.30	---	---	---	---	18.37	---	---	---	---
750 Gy	17.83	0.01	0.01	25.00	0.04	18.87	1.33	0.01	0.25	0.01
1000 Gy	20.40	0.05	0.04	80.00	0.42	22.07	1.35	0.02	1.73	0.01
1250 Gy	15.20	0.02	0.01	50.00	0.13	14.80	1.33	0.01	0.75	0.01
0.5% EMS	18.60	0.02	0.01	50.00	0.13	19.33	1.33	0.01	0.25	0.01
1.0% EMS	19.87	0.01	0.01	25.00	0.04	20.30	1.33	0.01	0.75	0.01
1.5% EMS	14.63	0.03	0.02	70.00	0.28	14.23	1.35	0.02	1.73	0.01
Shiralee										
Control	18.30	---	---	---	---	19.10	---	---	---	---
750 Gy	18.87	0.01	0.01	25.00	0.04	20.50	0.02	0.01	50.00	0.13
1000 Gy	19.50	0.02	0.01	50.00	0.13	21.17	0.01	0.01	25.00	0.04
1250 Gy	13.37	0.03	0.02	70.00	0.28	13.10	0.02	0.01	50.00	0.13
0.5% EMS	19.10	0.02	0.01	50.00	0.13	10.50	0.02	0.01	50.00	0.13
1.0% EMS	18.53	0.01	0.01	25.00	0.04	19.80	0.02	0.01	50.00	0.13
1.5% EMS	13.80	0.02	0.01	50.00	0.13	13.50	0.02	0.01	50.00	0.13

(ve=Environmental variance, vg=genetic variance, (h^2 b.s) heritability percentage and G.A=genetic advance)

Mean comparison for number of pod per plant: The results for number of pods per plant are summarized in the (Figure.2). In Hyola-42 the 1000 Gy induced highest value (437.33) while 1.0% of EMS gave highest value (291.33) over than control (264.33) for this character while in case of Shiralee the highest value (410.53) was showed by 1000 Gy of Gamma rays where as 1.0% of EMS exhibited highest value (285.67) higher than control (305.40) in M2 generation. In Hyola-42 1000 Gy of Gamma rays induced highest value (452.90) for number of pods per plan while 1.0 % of EMS gave highest value (316.13) higher than control (275.57) but Shiralee showed highest value (396.47) by 1000 Gy of Gamma rays and 1.0 % of EMS Showed highest value (332.97) higher than control (313.70) in M3 generation. Among all the applied doses of Gamma rays and EMS, The 1000Gy of gamma rays gave highest mean value (423.93) for number of pod per plant in M2 generation and same dose showed highest mean value while (424.68) in M3 generation. Shiralee has highest grand mean value (284.48) than Hyola-42 (272.05) in M2 and in M3 generation Shiralee has highest grand mean value (301.82) than Hyola-42 (273.53) for the number of pods per plant.

Results of M2 generation regarding the number of pods per plant (Figure.2) in canola genotypes indicated that medium dose of gamma rays and EMS effectively produced maximum number of pods per plants for both the genotypes. However, the minimum and maximum concentration of EMS and gamma rays did not give the best result in Hyola-42 and Shiralee.

The nearly similar results were also matched by the work of (Bolbhat *et al.*, 2012). in Horsegram (*Macrotyloma uniflorum* (LAM.) verdc) by using Gamma radiations and EMS that induced the variation for increasing the number of pods per plants at medium dose of Gamma rays 300Gy and 0.2% EMS. According to the findings in M3 generation (Table 5) the same trend was observed like minimum and higher dose of Gamma rays and EMS, did not gave the improvement for character in Hyola-42 genotype. However, in Shiralee only minimum doses of Gamma and EMS increased the pod that proved best response of Shiralee. Another research on Brassica presented that the mutagenic treatments up to medium dose of 1000 Gy improved the character and higher dose negatively affected for pods per plant in M2 and M3 generations (Emrani *et al*,. 2012).

In the findings of some other researchers in Brassica and other crops like Blackgerm; the maximum doses decrease the value, the genetic variability observed an increase in the number of pod per plant with moderate mutagen treatments (Arulbalachandran *et al*., 2010; Hussein *et al*,. 2012).

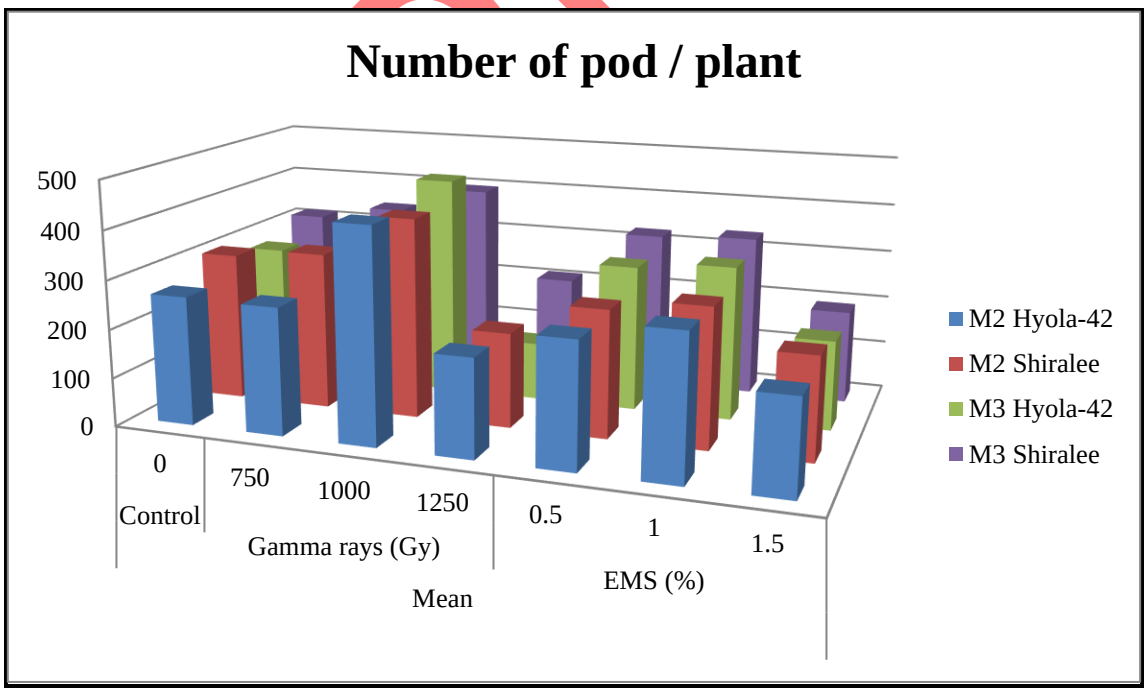

Figure 2. Gamma rays and EMS induced genetic diversity for Number of pod per plant in Canola species (Hyola-42 and Shiralee) in M2 and M3 generation.

Table 4. Genetic parameters of M2 and M3 Canola genotypes (Hyola-42 and Shiralee) affected by Gamma rays and EMS for Number of Pod / plant.

Treatments	M2					M3				
Hyola-42	**Mean**	**v.p**	**v.g**	**h^2**	**G.A**	**Mean**	**v.p**	**v.g**	**h^2**	**G.A**
Hyola-42										
Control	264.33	---	---	---	---	275.57	---	---	---	---
750 Gy	261.27	245.35	0.65	0.27	0.01	266.93	583.42	276.57	47.41	20.50
1000 Gy	437.33	364.15	119.45	32.80	9.32	452.90	336.03	29.19	8.69	1.22
1250 Gy	201.40	246.17	1.48	0.60	0.02	119.27	306.87	0.02	0.01	0.01
0.5% EMS	255.76	252.30	7.60	3.01	0.22	300.27	337.66	30.81	9.13	1.32
1.0% EMS	291.33	440.75	196.05	44.48	16.19	316.13	392.34	85.49	21.79	5.24
1.5% EMS	192.93	278.71	34.01	12.20	1.85	183.67	335.50	28.65	8.54	1.19
Shiralee										
Control	305.40	---	---	---	---	313.70	---	---	---	---
750 Gy	322.93	53.73	53.73	78.93	13.37	342.23	11.07	1.86	16.83	0.60
1000 Gy	410.53	14.29	2.97	20.80	0.93	396.47	40.62	31.41	77.33	11.27
1250 Gy	194.73	81.89	70.57	86.18	18.82	210.53	96.90	87.69	90.50	22.03
0.5% EMS	261.47	1081.13	1069.81	98.95	84.15	324.43	27.13	17.92	66.06	7.27
1.0% EMS	285.67	139.97	128.65	91.91	27.11	332.97	11.07	1.86	16.83	0.60
1.5% EMS	210.60	112.16	100.84	89.91	23.47	192.40	11.89	2.68	22.54	0.96

(ve=Environmental variance, vg=genetic variance, (h^2 b.s) heritability percentage and G.A=genetic advance)

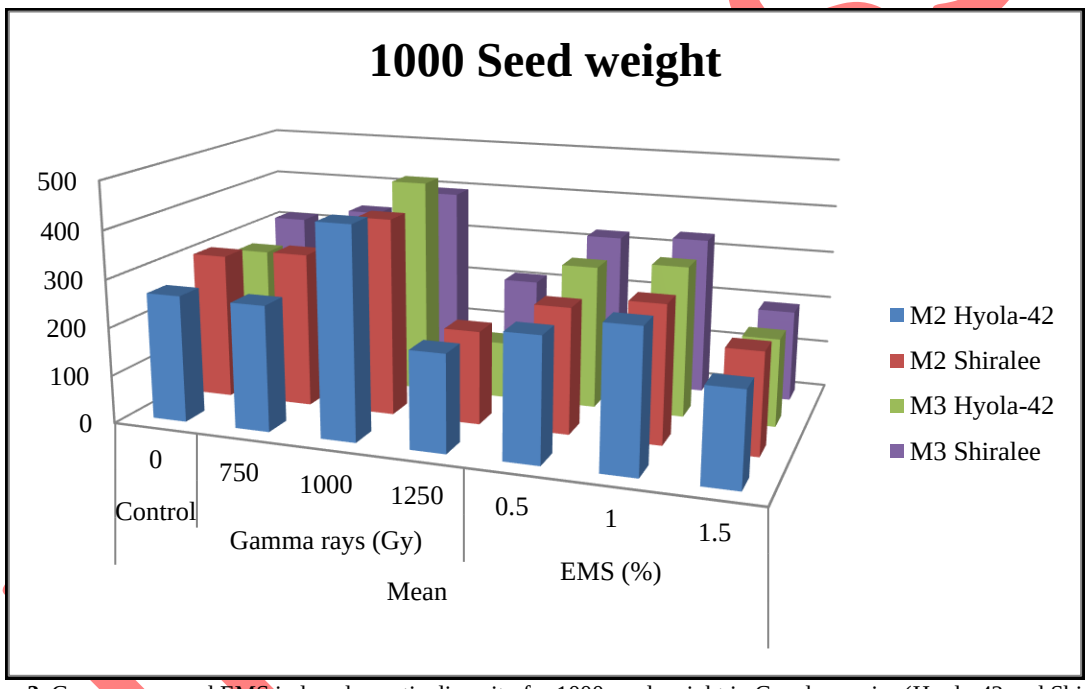

Figure 3. Gamma rays and EMS induced genetic diversity for 1000 seed weight in Canola species (Hyola-42 and Shiralee) in M2 and M3 generation.

Table 5. Genetic parameters of M2 and M3 Canola genotypes (Hyola-42 and Shiralee) affected by Gamma rays and EMS for 1000 seed weight.

Treatments	M2					M3				
	Mean	**v.p**	**v.g**	**h^2**	**G.A**	**Mean**	**v.p**	**v.g**	**h^2**	**G.A**
Hyola-42										
Control	2.34	---	---	---	---	2.49	---	---	---	---
750 Gy	2.56	0.01	0.01	36.21	0.02	2.79	0.01	0.01	26.92	0.02
1000 Gy	3.23	0.01	0.01	63.00	0.08	4.24	0.01	0.01	40.62	0.04
1250 Gy	1.28	0.01	0.01	74.66	0.12	1.10	0.01	0.01	79.79	0.21
0.5% EMS	2.88	0.01	0.0	42.19	0.03	3.13	0.01	0.01	56.82	0.09
1.0% EMS	2.75	0.01	0.01	56.98	0.06	3.40	0.01	0.01	79.79	0.21
1.5% EMS	1.06	0.01	0.01	36.21	0.02	1.07	0.01	0.01	56.82	0.09
Shiralee										
Control	2.62	---	---	---	---	2.86	---	---	---	---
750 Gy	2.47	0.01	0.01	35.00	0.01	3.11	0.01	0.01	95.31	0.22
1000 Gy	3.07	0.01	0.01	80.00	0.09	3.67	0.02	0.02	98.31	0.39

1250 Gy	1.55	0.01	0.01	18.75	0.00	1.33	0.01	0.01	89.29	0.13
0.5% EMS	2.24	0.01	0.01	48.0	0.02	2.53	0.01	0.01	89.29	0.13
1.0% EMS	2.95	0.01	0.01	68.29	0.05	3.08	0.01	0.01	25.00	0.01
1.5% EMS	1.19	0.01	0.01	18.75	0.00	1.17	0.01	0.01	89.29	0.13

(ve=Environmental variance, vg=genetic variance, (h^2 b.s) heritability percentage and G.A=genetic advance)

Heritability analysis for Number of pods per plant: In M2 generation 1.5% of EMS showed moderate heritability (h^2=44.48%) coupled with highest genetic advance (GA=16.19) in M2 generation while in M3 generation the moderate heritability (h^2=47.41%) coupled with highest genetic advance (GA=20.50) observed at 750 Gy in Hyola-42. In Shiralee the highest heritability in broad sense (h^2=98.95%) coupled with highest genetic advance (GA=84.15) observed at 0.5% EMS in M2 generation and high heritability (h^2=90.50%) with highest genetic advance (GA=22.03) was recorded at 1250 Gy in M3 generation for number of pods per plant (Table.4).

It was obvious from result that maximum number of pods per plant were produced by moderate dose of gamma rays. The important character, pods per plant showed moderate heritability couple with very high genetic advance for Hyola-42 in M2 generation at medium dose of EMS but with slightly improvement, moderate heritability and very high genetic advance observed in M3 generation. Shiralee produced high heritability combined with very high genetic advance in M2 by lower dose of EMS and high dose of gamma rays in M3 generation. Similar results were found by (Khan *et al.,* 2014).

Mean comparison for 1000 seed weight: The findings of this trait organized in the (Figure.3). In Hyola-42 Gamma rays produced highest 1000 seed weight value (3.2300g) at 1000 Gy while 0.5% of EMS gave highest value (2.8800g) greater than control (2.3433g) while in case of Shiralee 1000 Gy of Gamma irradiation induced highest value (3.0667g) and 1.0% of EMS gave highest value (2.9467g) than control (2.6233g) in M2 generation. In M3 generation Gamma rays generated highest value (4.2433g) of 1000 seed weight at 1000 while 1.0 % of EMS gave highest value (3.4000g) in Hyola-42 higher than control (2.4867g) and in case of Shiralee 1000 Gy of Gamma irradiation have highest value (3.666g) and 1.0% of EMS gave highest value (3.0833g) higher than control (2.8600) in M3 generation for this character. Among all the applied doses of EMS and Gamma rays, 1000 Gy gave highest value (3.1484g) for both the tested genotype in M2 generation and same dose 1000 Gy gave highest mean value (3.9550g) for both the tested genotype in M2 generation. Hyola-42 has highest grand mean value (2.3014g) than Shiralee (2.2986g) in M2 and Hyola-42 has highest grand mean value (2.6024g) than Shiralee (2.5357g) in M3 generation for 1000 seed weight.

The conclusion (Figure.3) based on applied doses of gamma rays and EMS in M2 generation of canola genotypes the Hyola-42 and Shiralee revealed that maximum values for 1000 seed weight, recorded at the medium dose of gamma rays 1000 Gy, but Hyola-42 genotypes proved to be most inductive for 1000 seed weight character.

The similar results were observed by the application of Gamma rays for 1000 seed weight in canola (*Brassica napus*) RGS003 genotypes. The maximum results were recorded by 1000Gy in both generations that proved effectiveness in inducing the highest genetic variability (Emrani *et al.,* 2012).

Significant differences observed for all treatments in both the genotypes in M3 generation concerning the 1000 seed weight (Figure.3). It was observed that minimum doses of Gamma radiations and EMS induce high value than control but maximum doses induced low values whereas moderate dose of Gy and EMS gave best results. Towards all treatments of Gamma rays and EMS, Hyola-42 showed an effective response for 1000 seed weight.

Results revealed that the highest doses of EMS and Gamma rays mutagenic treatments were negatively influenced for the trait of 1000 seed weight in both generations M2 and M3. The deleterious effect of the maximum dose of mutagen could be explained by the increased damage of cell constituents at molecular level or altered enzyme activity (Siddiqui *et al.,* 2021). The similar results were also conformed the work of other researchers for same trait (Emrani *et al.,* 2012).

Heritability analysis for 1000 seed weight: In Hyola-42 the evaluation for 1000 seed weight showed high (h^2=74.66%) with low genetic advance (GA=0.12) at 1250 Gy in M2 generation while moderate heritability (h^2=79.79%) along with low genetic advance (GA=0.21) observed at 1250 Gy and 1.0% EMS in M3 generation. In Shiralee the high heritability in broad sense (h^2=80.00%) with lowest genetic advance (GA=0.09) observed at 1000 Gy in M2 generation and highest heritability (h^2=98.31%) with lowest genetic advance (GA=0.39) was recorded at 1000 Gy in M3 generation for 1000 seed weight (Table.6).

Moderate heritability and low genetic advance (Table.6) observed in both genotypes Hyola-42 and Shiralee for moderate dose of gamma rays. In M3 generation moderate heritability with very low genetic advance observed in Hyola-42 at highest dose of gamma rays but highest heritability and low genetic advance found in Shiralee of M3 generation in medium dose of gamma rays due to presence of dominancy of gene or additive gene effect. Similar results were reported by (Siddiqui *et al.,* 2009; Uzair *et al.,* 2016; Shah *et al.,* 2021).

Mean comparison for seed yield per plant (g): The data for yield/plant (g) is exhibited in the (Figure.4). Gamma rays have highest seed yield value (11.530g) at 1000 Gy in Hyola-42 where as 1.0 % of EMS gave highest seed yield value (10.077g) higher than control (8.923g) where as in case of Shiralee, the 1000 Gy of

Gamma rays produced highest seed yield value (10.513g) and 0.5 % of EMS gave highest value (9.717g) than control (8.373g) in M2 generation. In Hyola-42, Gamma rays showed highest yield/plant value (12.600g) by 1000 where as 1.0 % of EMS gave highest seed yield (11.300g) than control (9.533g) while in case of Shiralee, the 1000 Gy of Gamma rays generated highest seed yield value (11.799g) and 1.0 % of EMS gave highest value (10.360g) than control (9.300g) in M3 generation. Among all the doses of EMS and Gamma rays the 1000 Gy of gamma rays gave highest mean value (11.022g) in M2 generation and 1000 Gy gave highest mean value (12.200) for seed yield/pant for both the tested genotypes in M3 generation. Over all Hyola-42 gave highest grand mean value (8.5138g) than Shiralee (8.3900g) in M2 and same genotype Hyola-42 gave highest grand mean value (8.8500g) than Shiralee (8.7290g) in M3 generation.

Significant differences in M2 generation were observed amongst all the applied doses of gamma rays and EMS for the Seed yield / plant under evaluation (Figure.4). Result indicated that minimum doses of Gamma rays and EMS gave high value but moderate doses were found best for both genotypes where as higher doses of Gamma and EMS gave very low value over the control in M2 and M3 generation. Hyola-42 found best responded towards the applied doses in both years.

Current findings were supported by the results of many earlier workers (Emrani *et al.*, 2012; (Shah *et al.*, 2021; Siddiqui *et al.*, 2021), who recorded differential effect of physical and chemical mutagen to improve seed yield per plant. Optimum results for gamma rays was obtained in1000 Gy dose and in both generations and cultivars.

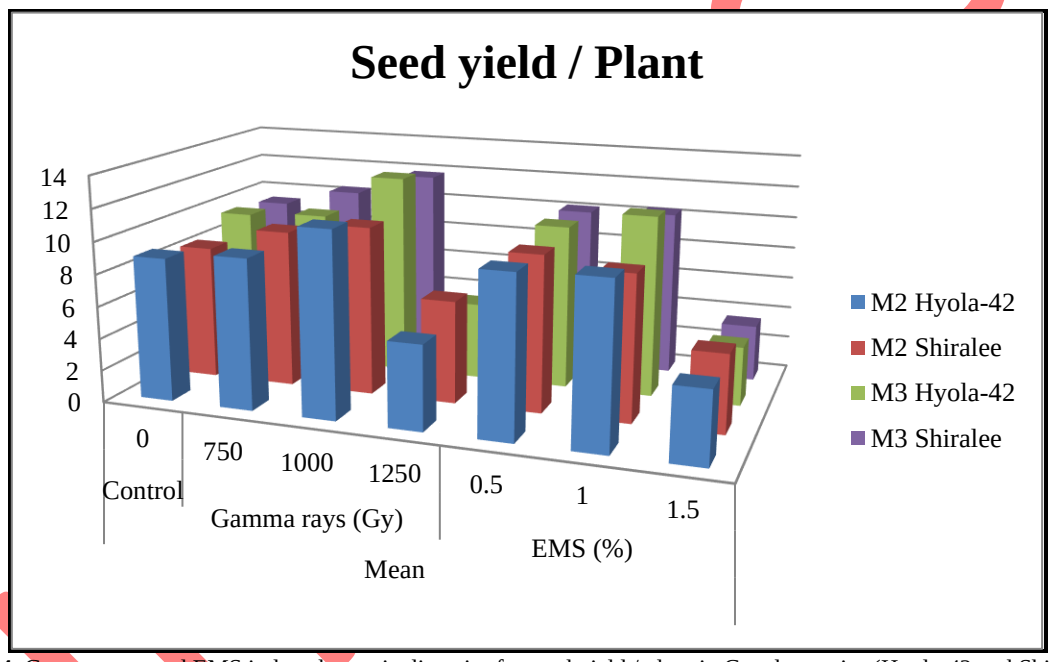

Figure 4. Gamma rays and EMS induced genetic diversity for seed yield / plant in Canola species (Hyola-42 and Shiralee) in M2 and M3 generation.

Table 6. Genetic parameters of M2 and M3 Canola genotypes (Hyola-42 and Shiralee) affected by Gamma rays and EMS for Seed Yield /plant.

Treatments	M2 Mean	v.p	v.g	h^2	G.A	M3 Mean	v.p	v.g	h^2	G.A
Hyola-42										
Control	8.92	---	---	---	---	9.53	---	---	---	---
750 Gy	9.38	0.02	0.01	49.23	0.10	9.80	0.01	0.01	75.00	0.20
1000 Gy	11.53	0.02	0.01	37.03	0.06	12.60	0.01	0.01	75.00	0.20
1250 Gy	5.26	0.01	0.01	19.43	0.02	4.80	0.01	0.01	75.00	0.20
0.5% EMS	9.94	0.01	0.01	19.76	0.02	10.25	0.01	0.01	21.87	0.20
1.0% EMS	10.08	0.02	0.01	23.46	0.03	11.30	0.01	0.01	75.00	0.19
1.5% EMS	4.48	0.01	0.01	5.69	0.00	3.67	0.01	0.01	2.91	0.01
Shiralee										
Control	8.37	---	---	---	---	9.30	---	---	---	---
750 Gy	9.82	0.01	0.01	26.92	0.02	10.40	0.02	0.01	50.00	0.13
1000 Gy	10.51	0.01	0.01	40.63	0.04	11.80	0.06	0.05	84.13	0.50
1250 Gy	6.39	0.01	0.01	65.92	0.12	5.57	0.03	0.02	70.00	0.28
0.5% EMS	9.72	0.01	0.01	14.61	0.01	10.17	0.01	0.01	7.69	0.01

1.0% EMS	9.06	0.01	0.01	44.53	0.05	10.36	0.01	0.01	13.79	0.01
1.5% EMS	4.86	0.01	0.01	40.63	0.04	3.51	0.01	0.01	2.28	0.01

(ve=Environmental variance, vg=genetic variance, (h^2 b.s) heritability percentage and G.A=genetic advance)

Heritability analysis for seed yield per plant: The heritability in broad sense (h^2=49.23%) estimated moderate with lowest genetic advance (GA=0.10) for seed yield / plant observed at 750 Gy in M2 generation and high heritability in broad sense observed (h^2=75.00%) with lowest genetic advance (GA=0.19) recorded at all the applied doses of gamma rays and EMS except 0.5% EMS and 1.5% EMS in M3 generation in Hyola-42. In case of Shiralee the moderate heritability in broad sense likely (h^2=65.92%) with low genetic advance (GA=0.12) calculated at 1250 Gy in M2 generation and high heritability in broad sense (h^2=84.13%) with lowest genetic advance (GA=0.50) showed by 1000 Gy in M3 generation for seed yield per plant (Table.6).

In M2 generation, moderate heritability and low genetic advance (Table.6) observed by moderate dose of EMS in Hyola-42 however Shiralee showed moderate heritability and low genetic advance induced by moderate dose of gamma rays in M3 generation. In both generations, the medium dose 1000 Gy gamma rays and Shiralee genotype exhibited the highest heritability and genetic advance in M3 generation. In the present study, M3 genotypes were more improved for this character than M2 generation. These improvements may be due to mutagenic induced changes at the genetic level. These results were confirmed by (Uzair *et al.*, 2016).

CONCLUSION

According to this comparative study M3 generation revealed improvement in yield associated characters by physical and chemical mutagen. Shiralee genotype produced maximum number of branches per plant and seed yield per plant due to best mutagenic effect of moderate dose of gamma rays. Number of pods per plant and 1000 seed weight were improved by moderate dose of gamma for Hyola-42.

AUTHOR CONTRIBUTION

Zeenat conducted the research, S.T, Qureshi and I. A, Khan planned and managed the experiment. R. A, Memon supported for writing the manuscript.

CONFLICT OF INTEREST

All the authors have no conflict on this publication.

(Supplementary files of images of crops in field have been uploaded)

REFRENCES

Addai, I. K. (2019). Selection in the M2 Generation of Soybeans (Glycine Max (L.) Merill) irradiated with Cobalt–60 Gamma Irradiation in the Guinea Savannah Agroecology of Ghana. Ghana Journal of Science, Technology and Development, **6**(1), 30-37.

Ali, H.M.A., and Shah, S. A. (2013). Evaluation and selection of rapeseed (*Brassica napus* L.) mutant lines for yield performance using augmented design. *Journal of Animal and Plant Science.* **23**: 1125–1130.

Amosova, A.V., Zoshchuk, S.A., Volovik, V.T., Shirokova, A.V., Horuzhiy, N.E., and Mozgova, G.V. (2019). Phenotypic, biochemical and genomic variability in generations of the rapeseed (*Brassica napus* L.) mutant lines obtained via chemical mutagenesis. *Public Library of Science*.(PLOS ONE)**14**(8):e0221699.

Anwar, M. M., Ali, S. E., & Nasr, E. H. (2015). Improving the nutritional value of canola seed by gamma irradiation. Journal of Radiation Research and Applied Sciences, **8**(3), 328-333.

Arulbalachandran, D., Mullainathan, L., Velu, S. and Thilagavathi, C. (2010). Genetic variability, heritability and genetic advance of quantitative traits in black gram by effects of mutation in field trail. *African Journal of Biotechnology*. **9**: 2731-2735.

Bolbhat, S.N., Gawade, B.B., Bhoge, V.D., Wadavkar, D.S., Shendage, V.S., and Dhumal K.N. (2012). Effect of mutagens on frequency and spectrum of viable mutations in Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). *Journal of Agriculture and Veterinary Science* (IOSR-JAVS) ISSN: 2319-2380. **1**,1(2012), PP45-55. www.iosrjournals.org. https://www.researchgate.net/publication/272712049.

Braatz, J., Harloff, H.J., Mascher, M., Stein, N., Himmelbach, A., and Jung, C. (2017). CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homologous gene copies in polyploidy oilseed rape (*Brassica napus*). *Plant Physiology*. **174** (2): 935–942.

Bus, A., Korber, N., Snowdon, R.J., and Stich, B. (2011). Patterns of molecular variation in a species-wide germplasm set of *Brassica napus*. *Theoretical and Applied Genetics,* **123**(8): 1413–1423.

Chaudhary, J., Deshmukh, R., and Sonah, H. (2019). Mutagenesis approaches and their role in crop improvement. Plants, 8 (11), 467

Emrani, S.M., Arzani, A., Saidi, G., Abtahi, M., Banifatimeh, M., Parsa, M.B. and Fatokian, M.H. (2012). Evaluation of induced genetic variability in agronomic traits by Gamma irradiation in canola (*Brassica napus* L.). *Pakistan Journal of Botany*. **44**(4): 1281 – 1288.

Emrani, S.N., Harloff, H., Gudi, O., Kopisch, Obuch, F., and Jung, C. (2015). Reduction in sinapine content in rapeseed (*Brassica napus* L.) by

induced mutations in Sinapine biosynthesis genes. *Molecular Breeding*, **35** (1): 37.

Falconer, D.S. (1977). Introduction to quantitative genetics. *Richard Clay Ltd. Bungay Suffolk, Great Britain* Pp.129-185.

Gupta, R., Mcroberts, R., Yu, Z., Smith, C., Saloan, W., and You, S. (2022). life cycle assessment of biodiesel production from rapeseed oil, inflorescence of process parameters and scale. *Bioresources Technology*, **360**-127532s.

Gupta, S., Kumar, A., Janeja, H.S., Prakash, A., and Anand, R. (2024). Genetic engineering in Indian Mustard (*Brassica juncea* L.): current progress and future directions for enhanced crop improvement. *Journal of Advances in Biology & Biotechnology*. **27**(5):739-751

Hasan, M., Seyis, F., and Badani, A.G. (2006). Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. *Genetics and Resources of Crop Evolution*, **53**(4): 793–802.

Hussain, S., Khan, W.M. and Khan, M. S. (2017). Mutagenic effect of sodium azide (NaN3) on M2 generation of *Brassica napus* L. (variety Dunkled). *Pure Applied Biology*, **6**: 226–236.

Hussein, O.S. (2012). Protein electrophoresis and DNA in herbs produced from irradiated Amrosia maritime seeds grown under soil salinity and their resistance to insects. American *Journal of Pant.Physiology.* **7**(6): 261-268.

Javeed, M.A., Khatri, A., Khan, I.A., Ahmed, M., Siddiqui, M.A., and Arain, A.G. (2000). Utilization of gamma irradiation for the genetic improvement of oriental mustard (*Brassia Juncea* Coss). *Pakistan Journal of Biological Sciences*, **32**(1)77-83.

Khan, W.M., Shah, S.Z., Khan, M.S., Islam, Z.U., Ali, S., Hussain, F., Irshad, M., and Zahid, M. (2014). Effects of gamma radiations on some morphological and biochemical characteristics of *Brassica napus* L. (variety Altex). *International Journal of Biological Sciences*. **4**(10):36-41, .

Larik, A.S., and Rajput, L.S. (2000). Estimation of selection indices in *Brassica junceae* L., *Brassica napus* L,. *Pakistan Journal of Biological Sciences*, **32**: 323-330.

Lee, Y.H., Park, W., and Kim, K.S. (2018). EMS-induced mutation of an endoplasmic reticulum oleate desaturase gene (FAD2-2) results in elevated oleic acid content in rapeseed (*Brassica napus* L.). *Euphytica* 214: 28.

Okuzaki, A., Ogawa, T., and Koizuka, C. (2018). CRISPR/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in *Brassica napus*. *Plant Physiology and Biochemistry*, **131**: 63–69.

Panda, S., Biswas, C. K., & Paul, S. (2024). Production of Biodiesel from Industrial Sludge: Recent Progress, Challenges, Perspective. Recent Trends in Management and Utilization of Industrial Sludge, 337-357.

Parry, M.A., Madgwick, P.J., and Bayon, C. (2009). Mutation discovery for crop improvement. *Journal of Experimental Botany* **60**: 2817–2825.

Singh, R.K., and Chandhri, B.D. (1985). Biometrical methods in quantitative genetics analysis. *Kalalyani Pub. Ludhiana. India*, Pp. 9-39.

Shah, S.A., Ali, I., and Rahman, K. (1990). Induction and selection of superior genetic variables of oilseed rape, *Brassica napus* L. *The Nucleus*, **7**: 37-40.

Sharafi, Y., Majidi, M.M., Goli, S.H., and Rashidi, F. (2015). Oil content and fattyacids composition in Brassica species. *International Journal of Food Propagation*. **18**: 2145–2154.

Siddiqui, M.S., Khan, I.A., and Khatri, A. (2009). Induced quantitative variability by Gamma rays and Ethylmethane sulphonate alone and in combination in rapeseed (*Brassica napus* L.). *Pakistan Journal of Biological Sciences*, **41**(3): 1189-1195.

Shah, M.A., Rehman, F.U., Mehmood, A., Ullah, F., Shah, S.I., and Rasheed, S.M. (2021).Combining ability, heritability and gene action assessment in rapeseed (Brassica napus L.) for yield and yield attributes. *Sarhad Journal of Agriculture*, **37**(1): 104-109.

Siddiqui, M.A., Aslam, M,M., Sial, M.A., Soomro, N.S., Khan, M.T., Yasmeen, S., Sial, S., and Khan, I.A. (2021). Surhan-2012: A novel high yielding, insect resistant, and high oil content canola variety released for general cultivation in the Sindh Province. *Pakistan Journal of Agricultural Research*, **34**(4):837-845.

STATIST (2021).

Uzair, M., Shahzadi, I., Jatoi, G.H., Bibi, T., Rauf, S., Mahmood, T., and Salah-ud-Din. (2016). Genetic variability and heritability studies in relation to seed yield and it's components traits in Mustard (*Brassica juncea* L.). *Academia Journal of Agricultural Research*, **4**(8): 478-482, DOI: 10.15413/ajar.2016.0177

Zeng, Q., and Liu, K. (2022). Worldwide rapeseed (*Brassica napus* L.) research: A bibliometric analysis during 2011–2021. *Oil Crop Science*. **7,** (4), Pp157-165.