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Original Research Article

OPTIMIZATION OF MUTAGEN DOSE FOR EARLY SEEDLING YIELD ASSOCIATED QUANTITATIVE CHARACTERS IN CANOLA GERM PLASM

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Abstract: *Pakistan is facing a vital shortage in the supply of health-promoting crops used as vegetables as well as oil sources. Brassica napus (Canola) is such a rich crop that occupies an important position in the oilseed crops of the world but unfortunately in Pakistan, its germplasm needs genetic improvement due to a narrow gene pool. Mutagenesis is a powerful tool for introducing new alleles, therefore different doses of Gamma radiations (750Gy, 1000Gy, 1250Gy) and EMS (0.5%, 1.0% and 1.5%) were used to induce genetic diversity for early yield-associated quantitative characters in two Canola genotypes (Hyola-41 and Shiralee). The data was recorded for days to flowering, days to maturity, plant height, number of branches per plant and pod length in M2 and M3 generation. Significant ($p \leq 0.05$) variations were observed for most of the phenotypic parameters for both mutagens. The mean value of all the phenotypic parameters in the M2 generation increased with a moderate dose of EMS and Gamma rays in both genotypes as compared to respective controls. Whereas in the M3 generation, both genotypes were more responsive to Gamma rays (1000 Gy) for all studied characters except early maturity in both tested genotypes. Heritability analysis exhibited high heritability and high genetic advance for most of the traits by moderate and highest doses of Gamma rays, while the reverse is true for EMS in both generations for Shiralee.*

Keywords: Mutagen, Yield, Quantitative Characters, Canola, Germ Plasm

Introduction

Brassica napus L., spp. *Olivera* commonly known as canola, rapeseed or oil seed is a biennial, economically important oil-producing herb as well as vegetable and condiment. Canola is one of the most important oil seed crops in the world (1) rapeseed and mustard is the second most important source of oil after cotton. *Brassica napus* is an exotic species that is originated in either the Mediterranean area or Northern Europe (2, 3) and introduced in South Asia in 5000 years BC (4) from Japan in 1934 (5).
The cultivation of rapeseed in Sindh, Pakistan dates back to 2600 and 1900 BCE in Moanjudharo (6). In Europe, rapeseed was grown commercially to be used as a lubricant with the invention of the steam engine in sixteen century. There is a long list of canola-growing countries (7). Pakistan Agriculture Research Counsel for the last two decades initiated comparative performance trials under local conditions on high-yielding exotic rapeseed cultivars such as Westar, Altex, Narendra, Shiralee and Hyola-42. The traditional consumption of canola as a meal and oil all around the world is diverse. As a vegetable canola green locally called Sag is tasty and famous to eat with rice and corn bread in Asia (8).
As the world's population increases day by day at an exponential rate, food safety measures are the main alarm of this 21st century. By 2050 world population is predicted to increase by 34% higher than today, which will reach up to 9.1 billion. To meet the demand of the increasing population there is a requirement of 70% more food production which puts massive pressure on the existing climate and agriculture (9-11). Demand for rapeseed oil for food and For Non-food uses, the production of hybrid cultivars with high seed and oil yields has become important in recent years (12).
The increasing demands for canola unitization created a great need for increased yield per hectare in terms of biomass and oil concentration. Different breeding options like varietal hybridization and artificial synthesis from the progenitor diploid species were practised with unsatisfactory outcomes due to a narrow gene pool. However, induced mutagenesis improved seed quantity and quality profiles remarkably (13-17). The oil quality character of rape and mustard follows a polygenic inheritance pattern (18). It could be achieved either by increasing the ploidy level (19, 20) or through gene mutations (21-23).
Among various physical mutagens, Gamma rays are usually the most effective mutagen and EMS is the most efficient among chemical mutagens for crop the improvement of qualitative traits. Few workers exploited these mutagens alone (24-26). Therefore better alleles generated through induced mutations in Canola to increase early yield associated traits of Canola have been done all around the world (16-18, 27, 28).
The degree of trait transmission from one to another mutation generation is more useful when integrated with genetic advance therefore heritability analysis of newly developed alleles is permissible for further breeding exploitation of genotypes (29, 30).
Therefore current study was undertaken to evaluate the relative effectiveness of mutagens and their doses were applied on two Canola (*Brassica napus* L.) varieties (Hyola-

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42 and Shiralee) in M2 and M3 mutant populations for **SIX** early yield associated quantitative traits.

Methodology

The 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 quantity and quality characters. The plants with improved characteristics will be used by plant breeders as parents in other crosses for variety development. The induced improvement in quantitative and qualitative character helps reduce protein malnutrition and canola essential oil. The increase in oil production will help in reducing the shortage of oil and its price for common people. The current study was undertaken the at Nuclear Institute of Agriculture (NIA), Tando Jam, Pakistan. Two best hybrid verities introduced from abroad Hyola-42 and Shiralee were selected for further genetic improvement. The seeds of these varieties were obtained from the Nuclear Institute of Agriculture (NIA) Tando Jam Sindh. The chemical mutagen, Ethyl Methane Sulphonate (EMS) (0.5 %, 1.0 % and 1.5 % and the physical mutagen, Gamma rays (750 Gy, 1000 Gy andas50 Gy) were applied, to improve early yields with associated Quantitative parameters of Canola. EMS treatments were carried out in the Plant Tissue Culture and Biotechnology Research Laboratory of NIA Tando Jam and Gama rays treatments were done at the Nuclear Institute of Medicine and Radiotherapy (NIMRA) Jamshoro. To compare the genetic improvement of each mutagen untreated seeds were used as a 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 distilled water (total 10 hrs). 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}. Mutagen-treated seeds (M0) of both varieties were sown in rows 4m long and 30 cm apart and plant to plant distance of 20cm to get M1, M2 and M3 generations in successive years. To raise each generation 5 pods from each terminal and primary racemes of each plant were harvested and bulked dose-wise for each treatment. Two rows of the control (untreated parent variety) were planted after every 20 rows of treated material to facilitate the comparison during selection. Selections for the desired early yield-associated traits were carried out on fifteen plants from each generation (M2 and M3) at random per treatment.

The data for the current work was recorded in M2 and M3 generations using five randomly selected plants from each line in every treatment. Six early yield-associated traits were scored for mean comparison and heritability analysis by following methods, the days were counted from sowing to 50% of plants developing flowers. Days were counted once again from the sowing date to the turning of the crop into golden yellow. Five randomly selected plants were measured from the ground soil level to the top using a meter scale in cm. Simple counting was done to record the number of branches per plant. The scale was used to score the length of pods in cm. Data of quantitative characters of M2 and M3 was statistically analyzed through Statistica version 8.1. Initially, a two-way Analysis of Variance (ANOVA) was done and further means were compared by the Least Significant Difference test at $p \leq 0.05$ (31).

The quantitative traits of M2 and M3 generation were further subjected to heritability analysis and compared with respective untreated control. Genetic parameters were calculated as suggested by (32) and (33).

Results

In this study two mutagens Gamma rays and EMS (Ethyl Methane Sulphonate) were applied to Canola genotypes: Hyola-42 and Shiralee for the analysis of quantitative characters. Evaluation of analysis of variances and mean comparison were achieved by DMRT (Duncan's Multiple Range Test). While compiling the results it relied on mean comparison but also evaluated up to Heritability measurements for the selection of a particular character.

ANOA and DMRT of early yield associated quantitative traits results of ANOVA (Table 1 & 2) for Quantitative characters i.e., days to flowering, days to maturity, plant height, number of primary branches/plant showed that varieties, treatments and varieties × Treatment are significantly different ($P \leq 0.05$) in both M2 and M3 generation. According to ANOVA pod length revealed that varieties are significant while treatments and varieties × treatment are non-significant ($P \leq 0.05$) in the M2 generation however varieties, treatments and varieties × Treatment are significantly different ($P \leq 0.05$) in the M3 generation of both the tested genotypes Hyola-42 and Shiralee in M3 generation.

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	Grand mean
	D. F	2	1	6	6	
1	Days to flowering	0.191	2.028	196.917	5.865	
2	Days to maturity	0.092	16.999	472.300	2.429	131.85
3	Plant height	0.301	27.088	383.326	2.685	150.14
4	Number of branched /plant	0.0435	8.3795	29.6784	0.4366	7.5843
5	Pod length	0.04690	0.46095	5.34976	0.14540	4.4324

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	Grand mean
	D. F	2	1	6	6	
1	Days to flowering	0.8405	7.697	208.458	1.762	72.179

2	Days to maturity	0.244	299.734	8.496	2.739	123.37
3	Plant height	0.437	4.314	529.699	0.151	150.91
4	Number of branched /plant	0.0559	1.4153	39.6112	1.1128	8.1083
5	Pod length	0.00655	0.00149	9.48787	0.12013	4.8902

Days to flowering mean comparison.

The means for days to flowering is summarized in (Fig 1). In Hyola-42 the highest value (83.717) was shown by 1.5% of EMS through 1250 Gy of Gamma rays induced (80.510) value higher than control (72.567) whereas in the case of Shiralee, 1.5% of EMS gave value (81.527) and 1000 Gy of Gamma rays induced value (78.367) higher than control (73.567) in M2 generation. The analysis of Hyola-42 for M3 generation indicated that 1250 Gy of Gamma rays induced the highest value (79.947) while 1.5% of EMS showed a value (79.467) higher than the control (70.747) whereas in the case of Shiralee, the 1250 Gy of Gamma rays produced highest value (80.257) for days to flowering and 1.5% of EMS gave value (81.847) higher than control (71.453) for same character. Among all the applied doses of Gamma rays and EMS, the 1.5% EMS gave the highest mean value (82.622) for both the tested genotype in the M2 generation and the same doses showed (80.657) value for the M3 generation. Shiralee has the highest grand mean value (73.392) than Hyola-42 (72.925) in M2 and (72.607) grand mean value in M3 generation which is higher than Hyola-42 (72.925).

The evaluated results of M2 generation for days to flowering indicated that among all the treatments of Gamma rays and EMS, the highest dose of EMS induced late days to flowering but the moderate dose of 1000 Gy of Gamma rays proved as the most effective treatment because it enhanced early flowering in both the genotypes with the comparison to the control. Overall, medium doses of Gamma rays and EMS induced early flowering in both the tested genotypes (Hyola-42 and Shiralee). Among the genotypes, Hyola-42 responded best to all the treatments of mutagens (Gamma rays and EMS) for days to flowering because it enhanced early flowering. Current findings are supported by the results of Emrani *et al*. (24), who found that 1000 Gy induced early flowering in both the *Brassica napus* cultivars. The cultivar RGS003 induced early flowering at 800 Gy and Sarigol at 1000 Gy treatments in M2 generations. Jagadeesan *et al*. (34) reported that mutants of two cultivars of sunflower have also decreased days to flowering due to treatment with 25 Krad gamma rays. Wongpiyasatid *et al*. (35) found an early flowering mutant in Mung Bean with the treatment of 500 Gy of gamma-ray. The significant effect of medium 25 Krad doses of Gamma rays on days to flowering in *Brassica napus* L. (variety Bulbul 98) was also confirmed by Khan *et al*. (36). It was also worker observed that a lower dose induces early flowering while the increase of dose delays flowering in comparison with control usually. He reported high genetic variation for days to flowering and described that days to flowering were mostly governed by additive gene effects (21).

Results of M3 generation indicated that EMS doses have a significant effect on days to flowering in both the genotypes (Hyola-42 and Shiralee) (Fig 1). The minimum and moderate concentration of EMS induced early flowering than control but the moderate dose of Gamma rays induced early flowering in Hyola-42 and Shiralee also. The response of Shiralee was best towards all the treatments of Gamma rays and EMS for days to flowering. Present results are consistent with the findings of Emrani *et al*. (24). They observed that lower and medium doses of gamma rays (800 Gy and 1000 Gy) induced early flowering in both of the tested canola varieties (RGS003 and Sarigol) compared to control. Khan *et al.* (36) also reported that days to flowering were postponed (110 days) at a higher dose (45 Krad) as compared to control (96 days) but treatment with 1200 Gy gamma-ray in M3, induced 7% earliness in flowering compared to control in Sarigol genotype. Jagadeesan *et al*. (34) also found decreasing trends for a number of days to flowering in different cultivars of sunflower due to treatment with 25 Krad gamma rays. An early flowering response in the genotype treated with a higher dose (500 Gy) of Gamma-ray was found in Mung bean (35). Early flowering is an essential agronomic character which ensures the saving of time and protects the yield loss from harsh environmental conditions which are mostly visible in late flowering crops. It has been an important character for estimation during a successful procreation program. Highly significant differences were observed among treated genotypes and control.

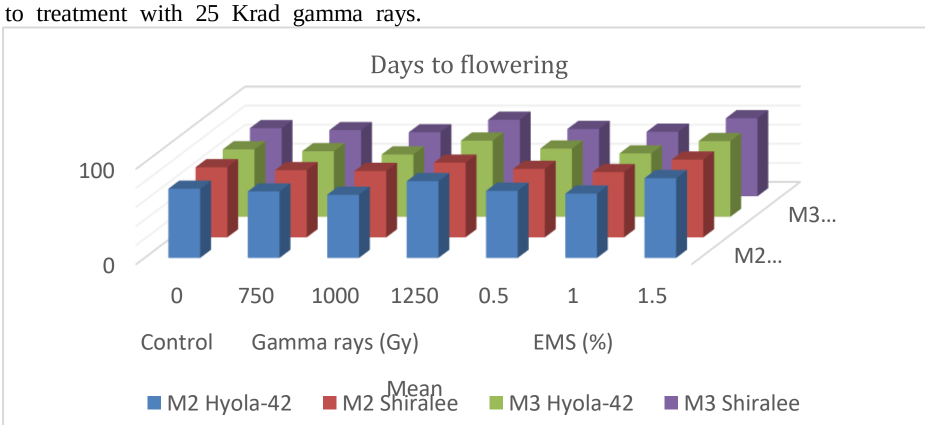

Fig 1. Gamma rays and EMS induced genetic diversity for days to flowering in Canola varieties (Hyola-42 and Shiralee) in M2 and M3 generation

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Heritability analysis

The high heritability in broad sense estimated (h^2 = 70.16%) with low genetic advance (GA = 0.70) showed by 1250 Gy in M2 generation while highest heritability in broad sense observed (h^2 = 99.19%) with low genetic advance (GA = 0.60) exhibited by 1.5% EMS in M3 generation in Hyola-42. Whereas in Shiralee, 1.0% of EMS showed moderate heritability in a broad sense (h^2 = 59.43%) with low genetic advance (GA = 0.66) at 1.0% of EMS in M2 generation and moderate heritability in a broad sense (h^2 = 56.62%) with low genetic advance (GA = 0.50) observed by1250 Gy in M3 generation for days to flowering (Table 3).

Table 3. Estimation of Genetic parameters of Canola varieties (Hyola-42 and Shiralee) affected by Gamma rays and EMS for Days to flowering in M2 and M3 generation

Treatments	M2					M3				
	Mean	v.p	v.g	h^2	G.A	Mean	v.p	v.g	h^2	G.A
Hyola-42										
Control	72.57	-----	-----	-----	-----	70.75	------	------	------	-------
750 Gy	69.70	0.11	0.05	42.49	0.24	68.74	0.00	0.00	50.00	0.03
1000 Gy	66.28	0.08	0.01	18.35	0.06	65.35	0.00	0.00	68.29	0.05
1250 Gy	80.51	0.21	0.15	70.16	0.70	79.95	0.00	0.00	59.38	0.04
0.5% EMS	70.41	0.20	0.13	67.69	0.64	71.55	0.00	0.00	68.29	0.05
1.0% EMS	67.48	0.20	0.14	68.95	0.67	66.45	0.00	0.00	48.00	0.02
1.5% EMS	83.72	0.17	0.11	62.83	0.53	79.47	0.05	0.05	99.19	0.60
Shiralee										
Control	73.57	------	------	------	------	71.45	------	------	------	------
750 Gy	70.44	0.21	0.9	41.37	0.32	69.35	0.01	0.01	50.00	0.04
1000 Gy	69.60	0.22	0.10	44.13	0.36	67.12	0.01	0.01	31.71	0.02
1250 Gy	78.37	0.24	0.12	49.24	0.44	80.26	0.01	0.01	56.92	0.05
0.5% EMS	71.78	0.15	0.03	19.14	0.09	70.56	0.01	0.01	30.00	0.02
1.0% EMS	68.46	0.30	0.18	59.43	0.66	67.66	0.01	0.01	49.09	0.04
1.5% EMS	81.53	0.19	0.07	35.58	0.24	81.85	0.01	0.01	36.36	0.02

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

Estimation of genetic advance and low values of heritability revealed that this character is controlled by a single gene. For the character of day-to-flowering (Table 3), high heritability with low genetic advance was recorded for higher doses of EMS in the M3 generation of Hyola-42, which indicated the non-additive gene effects due to fewer differences between means of control and treated genotypes, it was affected by environmental factors. Similar results were reported by Khan *et al*. (37). Moderate heritability and low genetic advance were recorded for Shiralee in high doses of Gamma rays in the M3 generation which can be used to estimate genetic transfer. The moderate genetic advance with low genetic advance was also considered by Khan *et al*. (38).

Days to maturity mean comparison.

The findings for days to maturity are assembled in Fig (2). In M2 generation the highest dose 1250 Gy showed the highest value (142.53) among gamma treatments while 1.5% of EMS induced the highest value (143.57) for Hyola-42 over the control (130.53) whereas in the case of Shiralee, the highest value was found at 1250 Gy (145.47) of gamma rays and 1.5% of EMS showed (144.43) value higher than control (132.42). In M3 generation, the 1250 Gy of gamma rays produced the highest value (141.88) and 1.5 % of EMS exhibited the highest value (141.34) higher than the control (129.26) for Hyola-42 whereas in the case of Shiralee the highest value was found at 1250 Gy (143.65) of gamma rays and 1.5% of EMS showed (142.49) days to maturity higher than control (130.72). Among all the applied doses of Gamma rays and EMS, the 1250 Gy of gamma rays induced the highest mean value (142.76) in M2 while in M3 generation 1250 Gy of gamma rays induced the highest mean value (144.00) for days to maturity for both tested genotype. Shiralee showed the highest grand mean value (132.49) than Hyola-42 (131.22) in the M2 generation although in the M3 generation, Shiralee exhibited the highest grand mean value (130.99) than Hyola-42 (129.85) for the days to maturity. Significant ($p \leq 0.05$) differences in M2 generation were observed in all the entries for the quantitative traits under evaluation. The result regarding the days to maturity (Fig 2) indicated that among all the treatments of Gamma rays and EMS the moderate concentration of EMS 1.0% induced early maturity as compared to the control and it was best for both the tested genotype (Hyola-42 and Shiralee) for inducing early maturity. Among the genotypes, Shiralee exhibited early maturity than Hyola-42 because it matured late. The closest results of present findings were observed by Khatri *et al.* (39) who developed three early maturing mutants of *Brassica juncea* L. cv. S-9 by exposing seeds to gamma rays and EMS. The lower dose of Gamma rays 750 Gy induced early maturity. In another work by Khan *et al*. (23), results revealed that increased concentrations of Gamma rays and EMS delay plant maturity, and early maturity is found at lower doses. In light of the present study, it is suggested to use a moderate dose of Gamma rays to obtain optimum early maturity in *Brassica napus* genotypes. The Gamma radiations treatments in the Sarigol cultivar induced early maturity in M2 and M3 generations reported by Emrani *et al*. (24). In the RGS003 cultivar the lower dose decreased the days to maturity and the higher dose increased the days to maturity in comparison with the control. In another work, rice found one line with 48 days earliness compared to control in M2 generation of seeds treated with 400 Gy gamma rays (40).

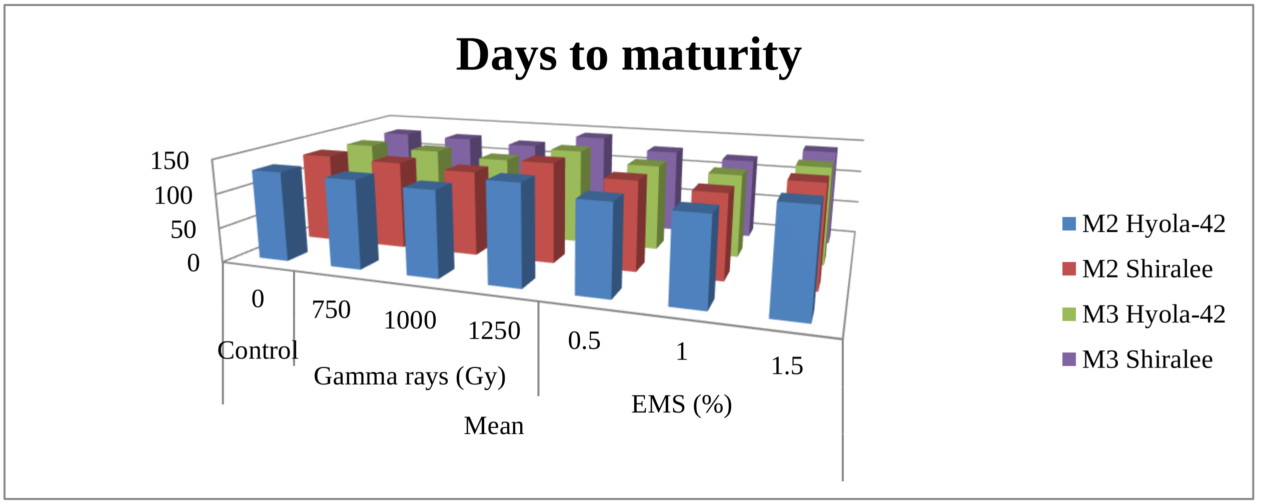

Fig 2. Gamma rays and EMS induced genetic diversity for the days to maturity in Canola varieties (Hyola-42 and Shiralee) in the M2 and M3 generation

In the M3 generation among all the mutants, a moderate dose of gamma rays was found to mature earlier but significantly earlier than the parent (Hyola-42 and Shiralee). Early maturity decreases the overall crop period in the field, consequently, it improves yield by minimizing the yield loss terminal heat stress during the seed development process. Results regarding the days to maturity (Fig 2) in canola-tested genotypes (Hyola-42 and Shiralee) revealed that Gamma rays at medium dose have effectively reduced days to maturity and induced early maturity. The tested genotype Hyola-42 responded best to all the treatments of Gamma radiation and EMS and matured earlier than Shiralee. Emrani *et al.* (24) also found that moderate Gamma is more effective in inducing early maturity in the 'Sarigol' cultivar in M2 (5.64%) and M3 (10.49 %) generations. They observed dose-dependent effectiveness in reducing days taken for maturity in the RGS003 cultivar as compared to the increase of doses in comparison with the control. Similar findings were recorded in rice for 400 Gy gamma rays treated seedlings that matured 48 days earlier than control (40).

Heritability analysis

For the days to maturity, moderate heritability in broad sense (h^2 = 65.72%) was observed with low genetic advance (GA = 0.72) at 1000 Gy in M2 generation while the highest heritability in broad sense observed (h^2 = 96.15%) with low genetic advance (GA = 0.25) by 1000 Gy in M3 generation in Hyola-42. However, in Shiralee, the 1000 Gy showed moderate heritability in the broad sense estimated (h^2 = 61.36%) with low genetic advance (GA = 0.85) in M2 generation and highest heritability in a broad sense (h^2 = 99.84%) with low genetic advance (GA = 0.99) observed by 1.5% of EMS in M3 generation for days to maturity (Table 4)

It was obvious from the results that high heritability with low genetic advance was recorded for Hyola-42 in moderate doses of gamma rays in M2 generation, controlled by additive gene effect. High heritability and high genetic advance were recorded for Shiralee in high doses of EMS in the M3 generation indicating the presence of dominancy of gene or heterozygosity and additive gene effect. Such results along with high heritability and high genetic advance were reported by Uzair *et al.* (41).

Table 4. Estimation of Genetic parameters of Canola varieties (Hyola-42 and Shiralee) affected by Gamma rays and EMS for Days to maturity in M2 and M3 generation

Treatments	M2					M3				
	Mean	v.p	v.g	h^2	G.A	Mean	v.p	v.g	h^2	G.A
					Hyola-42					
Control	130.53	---	---	---	---	29.26	---	---	---	---
750 Gy	128.24	0.16	0.07	43.35	0.30	127.15	0.01	0.01	70.00	0.06
1000 Gy	123.56	0.27	0.18	65.72	0.72	120.60	0.01	0.01	96.15	0.25
1250 Gy	142.53	0.14	0.04	31.71	0.17	141.88	0.01	0.01	52.00	0.03
0.5% EMS	127.41	0.17	0.08	46.66	0.35	126.84	0.01	0.01	70.00	0.06
1.0% EMS	122.67	0.17	0.07	43.87	0.31	121.92	0.01	0.01	52.00	0.03
1.5% EMS	143.57	0.12	0.02	20.00	0.08	141.34	0.01	0.01	70.00	0.06
					Shiralee					
Control	132.42	0.18	---	---	---	130.72	---	---	---	---
750 Gy	129.66	0.27	0.09	33.48	0.26	128.45	0.01	0.01	80.00	0.06

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1000 Gy	124.27	0.47	0.29	61.36	0.85	123.55	0.01	0.01	63.16	0.03
1250 Gy	145.47	0.22	0.04	19.63	0.11	143.65	0.01	0.01	80.00	0.06
0.5% EMS	129.53	0.26	0.08	30.88	0.23	127.34	0.01	0.01	50.00	0.02
1.0% EMS	121.63	0.22	0.04	18.36	0.10	120.76	0.01	0.01	75.00	0.05
1.5% EMS	144.43	0.22	0.04	19.38	0.10	142.49	0.01	0.01	99.84	0.99

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

Plant height means comparison.

The Data for plant height is summarized in Fig (3). As compared to the control (150.47cm), in Hyola-42, the 1000 Gy of gamma rays induced the highest plant height value (159.61cm) and 1.0% of EMS gave the highest value (154.35 cm) over the control (152.47) whereas in the case of Shiralee, Gamma rays induced highest plant height value (158.55 cm) at 1000 Gy of gamma rays and 1.0 % of EMS showed highest value (156.50 cm) that is higher than control (153.52 cm) in M2 generation. The comparison with the control, it is revealed that in Hyola-42, the 1000 Gy gamma rays showed the highest plant height value (160.73cm) and 1.0 % of EMS gave highest value (156.47 cm) above the control (153.62 cm) value while in case of Shiralee, 1000 Gy of Gamma rays induced highest plant height value (161.45 cm) and 1.0 % of EMS exhibited highest value (157.29 cm) than control (154.53 cm) in M3 generation. Among all the applied doses of Gamma rays and EMS, the 1000 Gy of gamma rays induced the highest mean value (159.08 cm) in M2 though in the M3 generation the same dose of 1000 Gy of gamma rays induced the highest mean value (161.09 cm) for plant height for both tested genotype ahyola-42 and Shiralee. In the M2 generation, Shiralee showed the highest grand mean value (150.94 cm) over the Hyola-42 (149.34) while in the M3 generation, the same variety Shiralee exhibited the highest grand mean value (151.23) greater than Hyola-42 (150.59) for the plant height. The analysis of the result of M2 generation regarding plant height in Fig (3) indicated that among all the treatments of Gamma rays and EMS, the highest treatment of EMS induced the shortest plant height in both genotypes, Hyola-42 and Shiralee as compared to control. The moderate doses of Gamma rays and EMS-induced heightened plants for both Hyola-42 and Shiralee than control. Based on evaluation the moderate dose of Gamma rays was best for both the tested genotypes (Hyola-42 and Shiralee). Among the genotypes, Shiralee found best responsive to all the treatments of Gamma rays and EMS for exhibiting the highest plant height. Similar results were found in the work of Khan *et al.* (36). He evaluated from the result that with the increase of dose, there is a reduction in the plant height. The highest doses 25 Krad, 35 Krad and 45 Krad significantly decreased the plant height and produced short-heighted plants. The reason behind the reduction in plant height can be endorsed in the mitotic activity of meristematic tissues and less amount of moisture in seeds as reported by Khalil *et al.* (42). The significant effects were noticed in lower doses of Gamma rays 750 Gy and higher concentration 1.0% of EMS on *Brassica juncea* cv. S-97 by (38). The three mutants were found to the short stature. It is evident from the result that plant height depends on available gene alleles and phenotypic response to their physiological and biochemical environment. This may be due to an increased rate of replication, cell division and differentiation that eventually altered the gene expression of growth hormones (43, 44). It can be inferred from current findings and previous work of mutation breeding of canola that multiple segment breaks induced through gamma rays and EMS cause chromosomal mismatch exchange known as translocations. At new locations, genes are regulated by the different gene promoters that are more active for mRNA synthesis resulting in over over-expression of gene product. This way many mutants with enhanced yield potential in rapeseed and mustard have been developed (45).

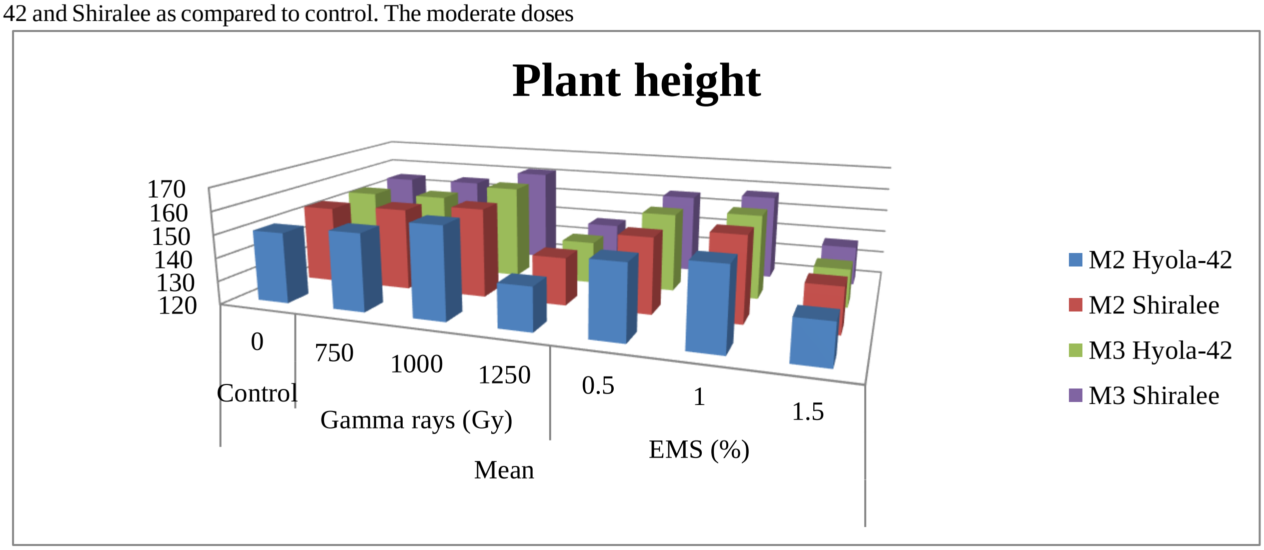

Fig 3. Gamma rays and EMS induced genetic diversity for plant height in Canola varieties (Hyola-42 and Shiralee) in M2 and M3 generation

Significant differences in M3 generation were observed amongst all the doses of chemical and physical mutagens for plant height under evaluation (Fig 3). All the mutants were induced to change in plant height but were significantly taller than parent Hyola-42 and Shiralee. All the mutants were differently affected by plant height but

[Citation Bano, Z., Qureshi, S.T., Memon, RA., Ahmed, I. (2024). Optimization of mutagen dose for early seedling yield associated quantitative characters in canola germ plasm. *Biol. Clin. Sci. Res. J.*, **2024**: *1194*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1194]

the maximum concentration of Gamma and EMS was responsible for producing short-stature plants in both the evaluated mutagen (Hyola-42 and Shiralee). With the comparison of both the mutagen varieties, the Shiralee responded best towards the doses of mutagens in producing the most desired character, the tall stature plants. Current findings are supported by the results of Siddiqui *et al*. (21) an increase in mutagen treatment decreases the plant height, Plant height was reduced in all the treatments except one i.e., 750 Gy+0.75% EMS. Current findings were consistent with the work of Khan *et al*. (23). The reduction in plant height at higher doses can be ascribed to mito-depression of meristematic due to enormous segment breaks leading to deletions of in many important genes required for active mitosis and cell differentiation as reported by Uzair *et al*. (41). Similarly, many researchers (46, 47) also noticed negative effect on the morphological characteristics such as plant height and number of branches of many crops treated with higher doses of gamma rays. The stimulating effect of low doses of gamma rays on plant growth may be due to less number of segment breaks that are rejoined at random to more active gene promoters than the original leading to stimulation of cell division or cell elongation, efficient metabolism, increased rate of phytohormones synthesis (42).

Heritability analysis

The evaluation for plant height showed that 1250 Gy of gamma rays produced moderate heritability in broad sense (h^2 = 40.15%) with low genetic advance (GA = 0.33) in M2 generation though high heritability in broad sense observed (h^2 = 73.87%) with high genetic advance (GA = 1.60) recorded at 1250 Gy of M3 generation in Hyola-42. In Shiralee the moderate heritability in a broad sense estimated (h^2 = 39.79%) with low genetic advance (GA = 0.31) by 1000 Gy of M2 generation and 1250 Gy showed the highest heritability in a broad sense (h^2 = 98.11%) with high genetic advance (GA = 2.12) 1250 Gy in M3 generation for plant height (Table 5). It was evident from the results (Table 5) that moderate heritability with low genetic advance was observed in the M2 generation for both genotypes, ahyola-42 and Shiralee but it was improved in the M3 generation for plant height in both genotypes. Similar results have also been reported by many work groups in Canada from other parts of the world (23, 48). The high and moderate heritability with high genetic advance was recorded in the M3 generation for both genotypes by higher doses of gamma rays because height is controlled by polygenes and when more dominant alleles are created via mutations and brought together in offspring it will result in heterosis.

Table 5. Estimation of Genetic parameters of Canola varieties (Hyola-42 and Shiralee) affected by Gamma rays and EMS for Plant height in M2 and M3 generation

Treatments	M2					M3				
	Mean	**v.p**	**v.g**	**h^2**	**G.A**	**Mean**	**v.p**	**v.g**	**h^2**	**G.A**
Hyola-42										
Control	150.47	---	---	---	---	153.62	---	---	---	---
750 Gy	153.43	0.18	0.03	17.76	0.08	154.21	0.53	0.29	54.06	0.75
1000 Gy	159.61	0.21	0.06	27.88	0.17	160.73	0.50	0.25	50.82	0.66
1250 Gy	138.56	0.25	0.10	40.15	0.33	138.47	0.94	0.69	73.87	1.60
0.5% EMS	151.57	0.16	0.01	5.10	0.01	154.07	0.34	0.09	27.57	0.22
1.0% EMS	154.35	0.18	0.03	16.11	0.07	156.47	0.30	0.05	17.87	0.11
1.5% EMS	137.36	0.21	0.06	29.34	0.19	136.53	0.34	0.09	27.57	0.22
Shiralee										
Control	153.55	---	---	---	---	154.53	---	---	---	---
750 Gy	155.56	0.16	0.02	14.62	0.06	154.93	0.34	0.33	96.08	1.43
1000 Gy	158.55	0.22	0.09	39.79	0.31	161.45	0.51	0.50	97.38	1.78
1250 Gy	140.52	0.18	0.05	25.66	0.14	138.44	0.71	0.69	98.11	2.12
0.5% EMS	152.45	0.20	0.06	31.17	0.20	154.87	0.43	0.41	96.87	1.62
1.0% EMS	156.50	0.19	0.05	28.27	0.17	157.29	0.34	0.32	96.03	1.42
1.5% EMS	139.50	0.16	0.03	17.78	0.08	137.07	0.43	0.41	96.87	1.62

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

Number of Primary branches per plant mean comparison.

The evaluated results for the primary branches per plant are assembled in Fig (4). In the M2 generation, the 1000 Gy of gamma rays showed the highest value (10.160) and 0.5% of EMS induced the highest value (8.107) in Hyola-42 as compared to the control (8.467), whereas the other genotype Shiralee showed the highest value at 1000 Gy (9.943) of gamma rays and 1.0% of EMS showed (9.183) reduced value to the control (9.613). In the M3 generation, the 1000 Gy of gamma rays exhibited the highest value (11.167) and 0.5 % of EMS showed the highest value (8.977) for Hyola-42 higher than the control (8.867) and in Shiralee, the same moderate dose of 1000 Gy of Gamma rays induced highest value (10.507) while 1.0% EMS showed highest value (10.250) inferior to the control (9.947) for this character. Among all the applied doses of Gamma rays and EMS, the 1000 Gy of gamma rays induced the highest mean value (10.052) in M2 although in the M3 generation, the same dose of 1000 Gy of gamma rays induced the highest mean value (10.837) for the number of primary branches per plant. Shiralee showed the highest grand mean value (8.0310) than Hyola-42 (7.1376) in the M2 generation although, in the M3 generation, Shiralee exhibited the highest grand mean value (8.2919) than Hyola-42 (7.9248) for the number of primary branches per plant is evident from the results (Fig 4) that highest number of branches per plants was achieved at the medium dose of

Gamma rays, lower and medium concentrations of EMS in Hyola-42 and a medium dose of Gamma rays induced in Shiralee for the highest number of primary branches per plant. Among the genotypes, Shiralee exhibited extreme response towards the treatments of Gamma rays and EMS as compared to Hyola-42. Current findings suggested that the increased number of branches per plant was dependent on specific genotypic responses to their physiological and biochemical environment. However, a similar effect of an increase in the number of branches was also observed by Yaseein and Aly *et al*. (48). They observed that in three tested genotypes of *Brassica napus* after the treatment with a higher dose of Gamma rays, there was a decrease in number of branches per plant in tested cultivars compared to control. Khan *et al.* (36) found in their research that medium doses of Gamma rays produced a maximum number of branches per plant on *Brassica napus* L. (variety Bulbul 98). The findings of the current work also confirmed that the applied lower and medium doses of Gamma rays induced a maximum number of branches in *Brassica napus* mutants (49, 23). A similar trend was also found in the work of (45, 50) in Horsegram for the number of branches per plant, The effect of gamma radiation on inducing diversity in branching may be due to its effect on the production and activation of growth hormones (51). Many other research groups working on mutation breeding of mung beans, lentils, buckwheat, and *Lepidium sativum*, observed a decrease in several branches with increasing doses of the mutagen (52, 53, 54, 55). Results of M3 generation indicated that a moderate dose of Gamma rays induced the maximum number of primary branches per plant in both the tested genotypes Hyola-42 and Shiralee. The moderate dose was best from all the applied doses and concentrations of Gamma rays and EMS. Shiralee responded best towards all mutagens. Current findings are supported by the results of (45, 56, 57, 23) (Fig 4).

Heritability analysis

In Hyola-42 the moderate heritability in a broad sense (h^2 = 57.10%) with low genetic advance (GA = 0.24) was calculated at 1000 Gy in the M2 generation although the highest heritability in a broad sense (h^2 = 99.15%) with high genetic advance (GA = 1.40) recorded at 1000 Gy in M3 generation. While in case of Shiralee, the moderate heritability in a broad sense likely (h^2 = 32.73%) with low genetic advance (GA = 0.10) evaluated by 1.5% of EMS in M2 generation and the highest heritability in broad sense (h^2 = 94.40%) with low genetic advance (GA = 0.42) was observed for 1250 Gy and 1.5% EMS in M3 generation for the number of primary branches/plant (Table 6).

It was manifested from the results that moderate heritability and low genetic advance were observed in the M2 generation and it was improved in the M3 generation for primary branches per plant in both the genotypes. High heritability coupled with high genetic advance was recorded for Hyola-42 in moderate doses of gamma rays in M2 generation, controlled by additive gene effect. The high heritability with high genetic advance recorded in the M3 generation for Shiralee by higher doses of gamma rays and EMS was observed. Current findings are consistent with those of Siddiqui *et al*. (21) in canola and Selvum *et al*. (58) in black gram for mean branch number against mutagen M2 population (Table 6).

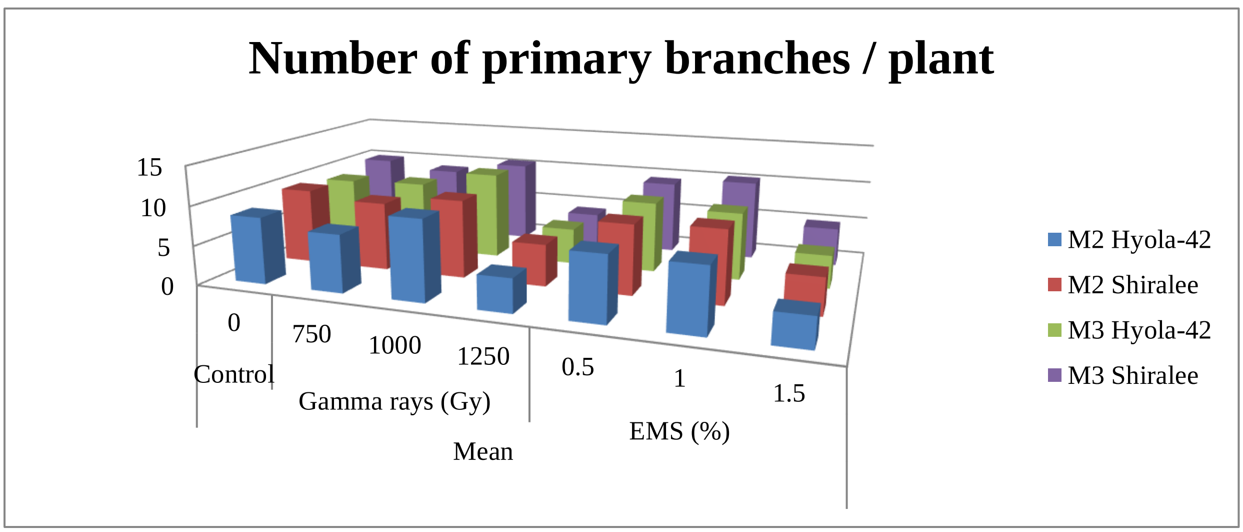

Fig 4. Gamma rays and EMS induced genetic diversity for several primary branches/plants in Canola varieties (Hyola-42 and Shiralee) in M2 and M3 generation

Table 6. Estimation of Genetic parameters of Canola varieties (Hyola-42 and Shiralee) affected by Gamma rays and EMS for the Number of Primary branches/plant in M2 and M3 generation

Treatments	M2					M3				
	Mean	v.p	v.g	h^2	G.A	Mean	v.p	v.g	h^2	G.A
Hyola-42										
Control	8.47	---	---	---	---	8.87	---	---	---	---
750 Gy	7.31	0.02	0.01	13.34	0.02	9.14	0.00	0.01	38.71	0.04
1000 Gy	10.16	0.05	0.03	57.10	0.24	11.48	0.30	0.29	99.15	1.40

[Citation Bano, Z., Qureshi, S.T., Memon, RA., Ahmed, I. (2024). Optimization of mutagen dose for early seedling yield associated quantitative characters in canola germ plasm. *Biol. Clin. Sci. Res. J.*, **2024***: 1194*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1194]

1250 Gy	4.23	0.03	0.01	25.62	0.06	4.60	0.28	0.28	99.10	1.36
0.5% EMS	8.11	0.03	0.01	26.43	0.06	8.98	0.02	0.01	83.30	0.24
1.0% EMS	7.97	0.02	0.00	0.49	0.00	8.55	0.00	0.01	26.92	0.02
1.5% EMS	3.72	0.02	0.00	9.17	0.01	4.18	0.03	0.03	90.94	0.38
Shiralee										
Control	9.61	---	---	---	---	9.95	---	---	---	---
750 Gy	8.73	0.04	0.01	28.05	0.08	8.97	0.01	0.01	7.14	0.01
1000 Gy	9.94	0.03	0.01	2.92	0.00	10.51	0.01	0.01	56.30	0.07
1250 Gy	5.30	0.04	0.01	24.37	0.06	4.28	0.01	0.03	94.40	0.42
0.5% EMS	8.81	0.03	0.01	5.00	0.01	9.36	0.01	0.01	26.76	0.02
1.0% EMS	9.18	0.03	0.01	9.44	0.01	10.25	0.01	0.01	42.86	0.04
1.5% EMS	4.64	0.05	0.02	32.73	0.10	4.28	0.03	0.03	94.40	0.42

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

Pod length (Cm) mean comparison.

The result for pod length is presented in Fig (5). In M2 generation gamma rays induced the highest pod length value (5.8357 cm) at 1000 Gy of gamma rays while 1.0% of EMS gave the highest value (4.9400 cm) which is advanced to control (4.2533 cm) in Hyola-42 in the case of Shiralee the 1000 Gy of gamma rays showed (6.4700 cm) highest value and 0.5 % of EMS exhibited (4.8767 cm) value over the control (4.6400 cm). In M3 generation Gamma rays induced the highest pod length at 1000 Gy (6.1733 cm) in Hyola-42 while 1.0% EMS gave the highest value (5.7800) above the control (4.7800 cm). Shiralee exhibited the highest value for pod length at 1000 Gy (6.3433 cm) whereas the highest value given by 1.0 % EMS was (5.9767 cm) over control (4.9200 cm). Among all the applied doses of Gamma rays and EMS, 1000 Gy of gamma rays showed the highest value (6.1533cm) in the M2 generation although in the M3 generation, the same dose of 1000 Gy of gamma rays induced the highest mean value (6.2583 cm) for pod length. Shiralee showed the overall highest grand mean value (4.5371 cm) for pod length than Hyola-42 (4.3276 cm) in the M2 generation while in the M3 generation, Hyola-42 exhibited the highest grand mean value (4.8962 cm) than Shiralee (4.8843).

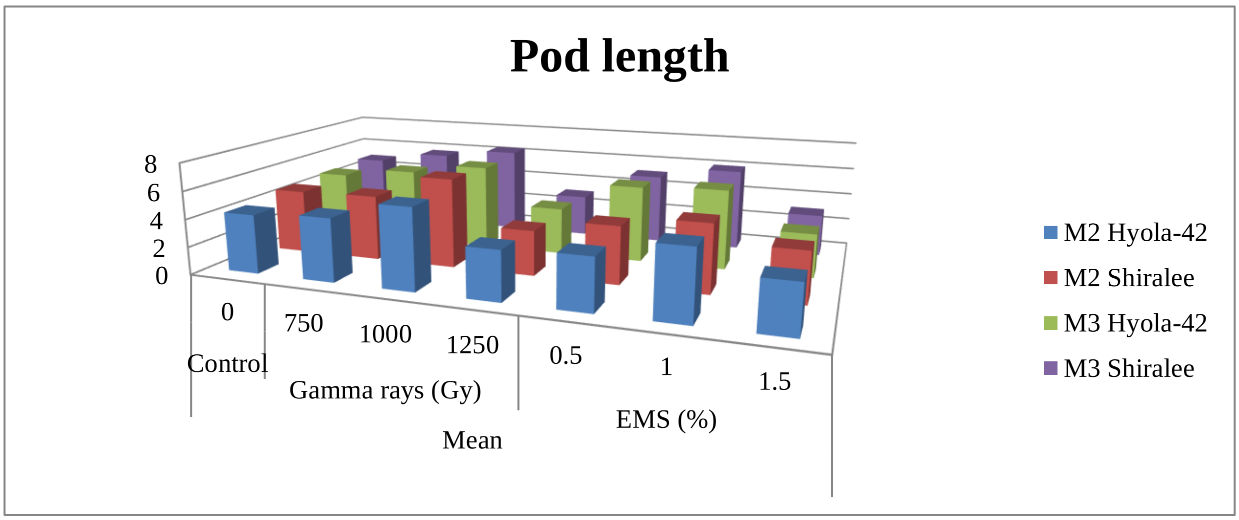

Fig 5. Gamma rays and EMS induced genetic diversity for pod length (cm) in Canola varieties (Hyola-42 and Shiralee) in M2 and M3 generation

The result of M2 generation regarding the pod length (Fig 5) indicated that among all the treatments of Gamma rays and EMS, the medium dose of gamma rays and EMS induced higher pod length than control for both the tested genotypes (Hyola-42 and Shiralee). Among the genotypes, Shiralee exhibited the highest pod length than Hyola-42 because Shiralee was found to respond best to all the treatments of Gamma rays and EMS. Current findings are supported by the results of Siddiqui *et al*. (21), who found maximum pod length at medium concentration (1.5%) of EMS in *Brassica napus*. A mutant with increased pod length (cm) has also been reported in rapeseed by Shah *et al*. (48). Other researchers have also reported nearly similar effects. The effect of Ethyl Methane Sulphonate and Diethyl Sulphate on chilli (*Capsicum annuum* L.) in M1 generation by using different concentrations of EMS 10 % to 50% EMS and from 5% to 25% DES by Gandhi *et al*. (59), found maximum pod length on the minimum treatment of EMS by 1.0% EMS. The analysis of M3 generation in Fig (5) indicated that the use of mutagens especially Gamma rays had significant effects on increasing pod length in Hyola-42 and Shiralee genotypes. A moderate dose of Gamma rays proved best in Hyola-42 followed by Shiralee caused a noticeable increase in pod length. Siddiqui *et al.* (21) found similar results, they found the highest value for primary branches per plant increased in combined treatments and separate treatments 1000 Gy.

Heritability analysis

[Citation Bano, Z., Qureshi, S.T., Memon, RA., Ahmed, I. (2024). Optimization of mutagen dose for early seedling yield associated quantitative characters in canola germ plasm. *Biol. Clin. Sci. Res. J.*, **2024**: *1194*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1194]

In the M2 generation, 0.5% of EMS showed the highest heritability (h^2 = 99.74%) coupled with high genetic advance (GA = 1.54) although moderate heritability (h^2 = 40.76%) with low genetic advance (GA = 0.10) was observed at 1000 Gy in M3 generation in Hyola-42 genotype. In the M2 generation, the highest heritability in a broad sense (h^2 = 99.87%) along with high genetic advance (GA = 1.45) was observed at 1.0% of EMS and moderate heritability (h^2 = 31.88%) with the lowest genetic advance (GA = 0.07) was observed at 1.5% EMS in M3 generation for pod length in Shiralee (Table 7). (ve=Environmental variance, vg=genetic variance, (h^2 b.s) heritability percentage and G.A=genetic advance)
One of the major seed yield-associated characteristics is pod length. Longer pod length produces more seeds. High heritability coupled with high genetic advance was recorded for Hyola-42 and Shiralee in low and moderate doses of EMS in M2 generation indicating the presence of dominancy of gene or additive gene effect. But Hyola-42 and Shiralee showed medium heritability with very low genetic advance observed in the M3 generation means heritability and genetic advance became high to low from M2 to M3 due to the repair of genes. Similar results were reported by Gandhi *et al.* (59). It is evident from the result that this gene is highly sensitive towered genetic changes and better allele may be evolved through point mutations induced by gamma radiation rather than double-stranded breaks resulting in translocation as a response of gamma rays. Mutagen treatment causes complex genetic and physiological damages (60).

Table 7. Estimation of Genetic parameters of Canola varieties (Hyola-42 and Shiralee) affected by Gamma rays and EMS for Pod length in M2 and M3 generation

Treatments	M2					M3				
	Mean	v.p	v.g	h^2	G.A	Mean	v.p	v.g	h^2	G.A
Hyola-42										
Control	4.25	---	---	---	---	4.78	---	---	---	---
750 Gy	4.55	0.01	0.01	40.43	0.03	5.43	0.02	0.01	28.46	0.05
1000 Gy	5.84	0.01	0.01	20.00	0.01	6.17	0.02	0.01	40.76	0.10
1250 Gy	3.54	0.01	0.01	30.00	0.02	3.42	0.01	0.01	1.85	0.01
0.5% EMS	3.72	0.36	0.35	99.74	1.54	5.54	0.01	0.35	16.40	0.02
1.0% EMS	4.94	0.01	0.01	30.00	0.02	5.78	0.01	0.01	0.80	0.01
1.5% EMS	3.45	0.01	0.01	20.00	0.01	3.15	0.01	0.01	7.00	0.01
Shiralee										
Control	4.64	---	---	---	---	4.92	---	---	---	---
750 Gy	4.74	0.01	0.01	72.09	0.06	5.73	0.04	0.02	5.85	0.20
1000 Gy	6.47	0.01	0.01	84.00	0.10	6.34	0.02	0.01	2.70	0.01
1250 Gy	3.25	0.01	0.01	63.64	0.04	3.06	0.02	0.01	2.50	0.01
0.5% EMS	4.14	0.01	0.01	50.00	0.03	5.13	0.02	0.01	1.47	0.01
1.0% EMS	4.88	0.31	0.31	99.87	1.45	5.98	0.02	0.01	9.48	0.01
1.5% EMS	3.64	0.01	0.01	50.00	0.03	3.03	0.02	0.01	31.88	0.07

Conclusion

1000 and 1250Gy gamma rays dose of physical mutagens and 1.0 and 1.5 % dose of EMS were more productive for most of the quantitative (high yield) and qualitative parameters (high oil content). Among tested genotypes Shiralee was more responded towards mutagens. Among mutagens, Gamma rays were more effective inducing positive mutations. Heritability analysis revealed that most of the characters were controlled by additive gene effect except seed/pod. Thus, genetic variability induced through mutation successfully improved early yield-associated traits.

Declarations

Data Availability statement
All data generated or analyzed for the selected five parameters during the study are included in the manuscript.
Ethics approval
Approved by the department concerned.
Consent for publication
Approved
Funding
Not applicable

Conflict of interest

The authors declared the absence of a conflict of interest.

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