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EVALUATION OF INDUCED GENETIC VARIABILITY IN AGRONOMIC TRAITS BY GAMMA IRRADIATION IN CANOLA (*BRASSICA NAPUS* L.)

SEYEDEH NAZGOL EMRANI [1*], AHMAD ARZANI[1], GODRATOLLAH SAEIDI[1], MOZHGAN ABTAHI[1], MOHAMMAD BANIFATEMEH[1], MOHAMMAD BAGHER PARSA[1] AND MOHAMMAD HOSSAIN FOTOKIAN[2]

[1]*Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, Iran*
[2]*Department of Agronomy and Plant Breeding, College of Agricultural Sciences, Shahed University, Tehran, Iran*
Corresponding author's e-mail: nazgol_6532@yahoo.com; Tel.: +98 311 391 3453; Fax: +98 311 391 2254

Abstract

In the present study, the induced variability caused by gamma rays in agronomic traits comprising plant height, days to flowering, days to maturity, number of fruits/plant, number of seeds/fruit, 1000-seed weight and seed yield/plant was investigated. Seeds of two canola cultivars ('RGS003' and 'Sarigol') were treated with 0, 800, 1000, 1200 Gy of gamma rays and the resultant M_2 and M_3 lines were grown under field conditions. Heritability (h^2), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and genetic advance of mean (GAM) were also estimated. The relationships between traits were also determined using correlation coefficient, path analysis and multiple stepwise regression analyses. Results of analysis of variance indicated highly significant effect of mutagenic doses, genotypes and genotype × dose interaction on the traits, indicating the differential response of genotypes to mutagenic treatments in terms of inducing genetic variations. The results revealed a higher variation in the treated populations than the control for all of the traits, with the highest GCV, PCV and GAM belonging to seed yield per plant. The highest variations induced with treatment of 1000 Gy of gamma rays in most of the traits in 'RGS003' cultivar, while 800 Gy gamma rays induced similar conditions in 'Sarigol' cultivar. The relationship between traits revealed major contribution of number of fruits per plant on justifying seed yield variation in both M_2 and M_3 generations. These results indicate that this yield component is the major determinants for fruit-yield differences among plants which also positively influences by irradiation mutagen (γ rays).

Introduction

Canola (*Brassica napus* L.) is an oilseed crop that has gained widespread acceptance worldwide due to the advantages of healthy edible oil and high oil yield. *Brassica* species are now the second largest oilseed crops after soybean in the global oilseed-crop production, surpassing peanut, sunflower and cottonseed during the last two decades (Anon., 2011). Oil containing only seven percent saturated fatty acids along with cholesterol-lowering monounsaturated and polyunsaturated fatty acids are the main components of canola seed oil (McDonald, 2011). Canola has been widely grown in Iran in recent years and a large number of genotypes, both winter and spring types, have been introduced from diverse sources in the world.

Exploiting natural or induced genetic diversity is a substantiated strategy in the improvement of all major food crops and the use of mutagenesis to create novel variation is particularly valuable in those crops with restricted genetic variability (Parry *et al*., 2009). In recent years, induced mutations have been extensively used for genetic enhancement of the annual oilseed crops, particularly in soybean, sesame, canola, sunflower and linseed (MacDonald *et al*., 1991; Ferrie *et al*., 2008; Velasco *et al*., 2008). Various mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens. The biological effect of ionizing radiation like gamma rays depends primarily on the amount of energy that will be absorbed by the biological system of which of course, the chromosomes are the most important target (Van Harten, 1998). It is known that irradiation of seeds increases mutation frequency and in turn promote gene recombination and widen the mutation spectrum (Larik *et al*., 2009). Worldwide efforts on mutation-based plant breeding have resulted in the official release to farmers of over 2700 new crop cultivars in approximately 170 species (Lagoda, 2008).

Development of high-yielding cultivars requires a thorough knowledge of the existing genetic variation for yield and its components. The observed variability is a combined estimate of genetic and environmental causes, of which only the former is heritable. However, estimates of heritability alone do not provide a full anticipation of the outcome in plant response to selection, but should be combined with other estimates such as genetic advance, the change in mean value between generations, phenotypic and genotypic coefficients of variation (Wani & Khan, 2006).

Average yield of canola can be improved up to its genetic potential which is much higher than the present status. Direct and indirect selection via yield components provides the basis for its successful breeding program and hence the yield-complexity problem can be more effectively tackled. Use of simple correlation analysis could not fully explain the relationships among the traits. Therefore, the path coefficient analysis has been used by many researchers for a more complete determination of the impact of independent variables on dependent one (Ali *et al*., 2003). The path coefficient analysis helps the breeders to explain direct and indirect effects and hence has extensively been used in breeding programs in different crop species by various researchers (Punia & Gill, 1994; Shalini *et al*., 2000; Ali *et al*., 2002). Stepwise multiple regression analysis is used to determine the contribution of more important traits that have significant association with seed yield (Golkar *et al*., 2011).

Genetic parameters to determine the selection criteria for yield improvement were investigated in rapeseed and mustard. Sheikh *et al.,* (1999) found high heritability estimates coupled with high genetic advance for seed yield per plant, primary and secondary branches, fruits per plant and seed weight in rapeseed genotypes. They also reported positive correlation of all the yield components with seed yield. Number of fruits per plant, number of seeds per fruit, harvest index and 1000-seed weight are known as the yield components in canola (Caliskan *et al.,* 1998).

Gamma and X rays have been extensively used to induce mutations in crop plants. Dose of irradiation of plant materials and the management of induced variations are critical factors in successful adoption of the technology (Ahloowalia & Maluszynski, 2001). However, such studies are lacking in *Brassica* oilseed crops. The objectives of present study are to assess the optimal dose γ rays to induce variation in yield and yield components and the relationships between yield and yield components using multivariate analyses in M_2 and M_3 lines of 2 canola cultivars.

Materials and Methods

Plant material and radiation treatment: Two spring canola cultivars 'RGS003' and 'Sarigol' have been used in this study. RGS003 was originated from Germany while 'Sarigol' is an Iranian cultivar. Seeds were irradiated with gamma radiation at Iranian Atomic Energy Center, Karaj, Iran. The seeds were treated at 0, 800, 1000 and 1200 Gy of gamma radiation from Cobalt-60 (^{60}Co) source.

Field experiments: The irradiated and control seeds were planted at Research Farm of College of Agriculture, Shahed University, in winter 2008. Seeds harvested from individual M1 plants randomly were sown in winter 2009 as M2 lines on 2 meter rows with 40cm space between single plants in a randomized complete block design with 3 replications at Research Farm of College of Agriculture, Isfahan University of Technology located in Lavark, Najaf-Abad, Iran (51º 32´ E and 32º 32´ N, 1630 m asl). For raising the M3 generation, 30 M2 lines were selected in each treatment which showed significant deviations in mean values in the positive direction from the mean values of the control. A similar planting procedure of M2 lines was applied for M3 lines.

Days to flowering (DF), days to maturity (DM), plant height (PH) (cm), number of fruits per plant (PP), number of seeds per fruit (SP), 1000 seed weight (SW) (g) and seed yield per plant (SY) (g) were recorded in M2 and M3 generations using five randomly selected plants from each line in every treatment. The data were averaged on the 30 M_3 lines belonging to each treatment were subjected to the statistical analyses.

Statistical analysis: Analysis of variance, simple phenotypic and genotypic correlation and stepwise regression analysis were performed using SAS program (Anon., 2008). Path coefficient analysis was carried out by SPSS Ver.9 using seed yield as dependent variable and the remaining traits as independent variables.
In order to evaluate the effects of the mutagenic treatments on inducing variation, the following parameters were calculated: phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (h²) and expected genetic advance as percentage to mean (GAM). The expected genetic advance for each trait with assumed 1% selection intensity was computed using the following equation according to Allard (1960):

$$GA = k \times \sigma_p \times h^2$$

$$GAM = (GA / mean) \times 100$$

Where: h² = broad-sense heritability, σp = phenotypic standard deviation of the mean performance of the treated population and k = 2.64, constant for 1% selection intensity.

Results and Discussion

Results of analysis of variance of the studied agronomic traits in M2 and M3 generations are summarized in Table 1. Gamma doses significantly affected the variations of all traits in both generations. Two canola cultivars differed significantly for all traits with the exception of number of seeds per fruit in M_2 and number of fruits per plant in M_3 generations. The cultivar × dose interaction was also highly significant for all the traits at both generations. Therefore, these cultivars were differentially affected by the irradiation doses in terms of induction of genetic variations.

Table 1. Results of analysis of variance for quantitative traits in M_2 and M_3 generations of *Brassica napus* L. affected by gamma irradiation doses.

Source of variation	df	Plant height	Days to flowering	Days to maturity	Number of fruit per plant	Number of seed per fruit	1000 seed weight	Seed yield
M_2 generation								
Replication	2	15.28**	0.29	1.20*	28.96	0.01	0.05**	0.87**
Cultivar	1	245.44**	735.93**	1044.12**	3201.89**	0.02	0.08**	1.14**
Dose	3	88.22**	30.18**	11.25**	880.48**	26.06**	1.27**	20.05**
Cultivar × Dose	3	253.34**	50.01**	58.70**	283.76**	17.31**	0.19**	1.89**
Error	14	1.74	0.23	0.29	11.59	0.06	0.002	0.03
M_3 generation								
Replication	2	10.19*	0.77	0.50	79.63*	0.40	0.005	1.36**
Cultivar	1	24.81**	1156.62**	2247.89**	57.75	2.37**	0.33**	26.10**
Dose	3	63.64**	8.48**	75.04**	5130.92**	7.77**	0.19**	22.05**
Cultivar × Dose	3	131.50**	22.35**	76.69**	359.22**	8.92**	0.20**	0.93**
Error	14	2.05	0.23	0.20	19.67	0.14	0.005	0.13

* and ** significant at 0.05 and 0.01 of probability levels, respectively

Genetic variability: Inducing variation via mutagenesis on quantitative traits can be inferred by the means and genetic parameters. Mutagenic treatments have increased the plant height for RGS003 in both generations, where mutant lines in 800 Gy-treatment had the highest mean values for plant height (Table 2). In the case of 'Sarigol', treatment with 1000 Gy gamma rays produced the lowest plant height in M_2 and M_3 generations. The highest phenotypic and genetic coefficients of variation, heritability and genetic advance for plant height were obtained at mutagenic treatment 1000 Gy in 'RGS003', while these were obtained for 'Sarigol' at 800 Gy-treatment. In canola, Siddiqui *et al.,* (2009) used 2 gamma ray doses (750 and 100 Gy) and 2 ethylmethane sulphonate (EMS) concentrations (0.75 and 1%) in 1 cultivar and found reduction of plant height with increasing gamma rays. They observed the highest amount of variability in this trait in the combined treatment of seeds with 1000 Gy gamma rays and 0.75% EMS.

Mutagen treatment has increased days to flowering in both cultivars compared to control in M_2 generation with the exception of RGS003-800 and Sarigol-1000 treatments (Table 2). Treatment with 1200 Gy mutagen induced the highest heritability of flowering date in both cultivars while treatment with 1000 showed the highest values for GCV, PCV and GAM in both cultivars. These results showed that 1000 Gy-treatment induced the highest amount of variation in M_2 generation. The disparity in the results could be due to the fact that heritability is a property not only of a character but also of the population and the environment to which the genotypes are subjected to. Hence, heritability alone is not a reliable parameter to predict the selection effect and high phenotypic and genotypic coefficient of variation indicate better chance for selection to be successful. Treatment with 1200 Gy gamma ray in M_3, induced 7% earliness in flowering compared to control in 'Sarigol'. Jagadeesan *et al.,* (2008) has also reported decreased number of days to flowering due to treatment with 25 KR gamma rays in mutants of two cultivars of sunflower. Wongpiyasatid *et al.,* (1998) found an early flowering mutant in mung bean treated with 500 Gy of gamma ray. In the present study, treatment with 1200 Gy mutagen induced the highest variability in M_3 for flowering date in both cultivars, considering high heritability, GCV, PCV and GAM. On the other hand, control possessed the lowest amount of these parameters in both cultivars at both generations.

Table 2. Means, phenotypic (PCV) and genetic (GCV) coefficient of variation, heritability (h2) and genetic advance over mean (GAM) at M_2 and M_3 generations of two cultivars of *Brassica napus* L. affected by gamma irradiation doses.

Treatments	M_2					M_3				
	Mean	h^2	GCV	PCV	GAM	Mean	h^2	GCV	PCV	GAM
Plant height										
RGS003-0	74.11^{c1}	58.61	5.04	6.59	10.19	81.29^{c}	76.30	6.84	7.84	15.79
RGS003-800	87.83^{a}	84.94	14.13	15.33	34.39	86.74^{a}	83.22	12.02	13.17	28.94
RGS003-1000	83.22^{b}	88.09	21.84	23.27	54.13	84.65^{b}	92.55	17.85	18.55	45.33
RGS003-1200	83.54^{b}	79.47	9.72	10.91	22.89	84.76^{b}	84.42	11.33	12.33	27.49
Sarigol-0	93.69^{b}	50.18	5.20	7.35	9.73	94.79^{a}	28.61	2.68	5.01	3.78
Sarigol-800	80.06^{c}	87.86	19.85	21.17	49.11	83.44^{c}	95.50	24.99	25.57	64.48
Sarigol-1000	81.99^{c}	81.61	12.67	14.03	30.22	77.12^{d}	87.99	11.83	12.61	29.30
Sarigol-1200	98.55^{a}	82.89	15.45	16.97	37.13	90.22^{b}	91.12	17.19	18.00	43.31
Mean	85.37	76.71	12.99	14.45	30.97	85.38	79.96	13.09	14.13	32.30
Days to flowering										
RGS003-0	67.43^{c}	31.14	1.04	1.87	1.54	67.14^{b}	58.68	1.40	1.83	2.84
RGS003-800	64.45^{d}	68.16	2.37	2.87	5.17	65.61^{c}	67.06	2.27	2.78	4.92
RGS003-1000	76.00^{a}	94.37	11.14	11.47	28.57	67.63^{b}	83.83	3.16	3.45	7.64
RGS003-1200	72.09^{b}	96.43	7.02	7.16	18.22	68.33^{a}	97.63	8.92	9.03	23.26
Sarigol-0	80.60^{b}	53.59	1.90	2.59	3.67	82.18^{a}	25.39	0.82	1.62	1.08
Sarigol-800	82.37^{a}	85.53	3.06	3.31	7.48	82.69^{a}	89.19	3.09	3.27	7.71
Sarigol-1000	80.52^{b}	64.51	9.61	11.97	20.39	82.79^{a}	89.79	4.08	4.31	10.21
Sarigol-1200	80.78^{b}	93.32	7.59	7.86	19.37	76.59^{b}	98.03	10.74	10.85	28.07
Mean	75.53	73.38	5.47	6.14	13.05	74.12	76.20	4.31	4.64	10.72
Days to maturity										
RGS003-0	119.59^{c}	28.54	0.61	1.13	0.85	119.07^{a}	51.86	0.79	1.10	1.50
RGS003-800	116.15^{d}	62.54	1.30	1.65	2.72	113.25^{c}	70.28	1.35	1.61	2.98
RGS003-1000	122.52^{b}	79.49	5.51	6.18	12.97	115.14^{b}	91.34	1.96	2.05	4.94
RGS003-1200	123.87^{a}	95.13	3.97	4.08	10.24	119.73^{a}	96.72	5.04	5.12	13.08
Sarigol-0	136.48^{a}	34.22	1.26	2.16	1.95	143.71^{a}	34.41	0.55	0.93	0.85
Sarigol-800	135.13^{b}	77.93	1.74	1.97	4.05	134.02^{c}	89.31	2.08	2.20	5.18
Sarigol-1000	134.52^{b}	56.03	4.42	5.90	8.73	138.25^{b}	91.75	2.47	2.58	6.25
Sarigol-1200	128.77^{c}	89.78	4.57	4.83	11.44	128.63^{d}	97.57	5.91	5.98	15.40
Mean	127.13	65.46	2.92	3.49	6.62	126.47	77.90	2.52	2.70	6.27

[1]Means in each column with a common letter are not significantly differed at $LSD_{5\%}$.

Gamma irradiation treatment induced early maturity in ‘Sarigol’ cultivar having days to maturity reduction of 5.64 and 10.49 % in M_2 and M_3 generations, respectively (Table 2). It is interesting to note that this trait followed different reaction to irradiations in RGS003 cultivar with declining at 800 Gy and increasing afterward with the increase of doses in comparison with control. The highest estimates of heritability were recorded for 1200 Gy-treatment for both cultivars and in both generations. Treatment with 1200 Gy gamma rays also induced the highest values of GCV, PCV and GAM in M_3 lines of both cultivars. On the other hand, 1000 Gy-treatment induced the highest values of GCV, PCV and GAM in M_2 lines of RGS003.This treatment has also induced the highest amount of variability for days to flowering in M_2 generation (Table 2). In the present study, 15 days earliness was induced by 1200 Gy in M_3 lines of ‘Sarigol’ canola cultivar. In rice, Shew & Shiaikh (1993) found one line with 48 days earliness compared to control in M_2 generation of seeds treated with 400 Gy gamma rays.

Number of fruits per plant in RGS003 was increased with mutagenic treatments up to dose of 1000 Gy (Table 3). Using 1200 Gy of gamma rays negatively affected this trait showing 25.23% and 19.91% reduction compared to the 1000 Gy treatment in M2 and M3 generations, respectively. Arulbalachandran *et al.,* (2010) studied the genetic variability induced by different doses of gamma rays in blackgerm. They observed an increase in the number of fruits per plant with increasing mutagen treatments up to 60 KR and the reduction of this trait afterward. The same trend has been shown in M_3 lines of ‘Sarigol’. Gamma rays dose of 800 Gy produced the highest coefficients of variability and genetic advance in both years with the exception of M_3 lines in RGS003.

Table 3. Means, phenotypic (PCV) and genetic (GCV) coefficient of variation, heritability (h2) and genetic advance over mean (GAM) at M_2 and M_3 generations of two cultivars of *Brassica napus* L., affected by gamma irradiation doses.

Treatments	M_2					M_3				
	Mean	h^2	GCV	PCV	GAM	Mean	h^2	GCV	PCV	GAM
Number of fruits per plant										
RGS003-0	107.73^{c}	48.40	15.13	21.75	27.78	131.84^{d}	66.72	12.81	15.69	27.63
RGS003-800	123.33^{b}	85.36	33.50	36.26	81.72	172.99^{b}	55.10	13.59	18.31	26.63
RGS003-1000	143.21^{a}	93.62	30.91	31.95	78.96	185.94^{a}	91.98	18.86	19.67	47.76
RGS003-1200	107.08^{c}	89.15	21.68	22.97	54.05	148.91^{c}	81.29	13.64	15.13	32.48
Sarigol-0	146.49^{b}	74.30	18.36	21.30	41.78	109.94^{c}	47.59	10.33	14.97	18.81
Sarigol-800	133.70^{c}	93.45	31.86	32.96	81.32	163.31^{b}	91.67	20.52	21.43	51.87
Sarigol-1000	156.02^{a}	91.33	29.26	30.61	73.82	195.42^{a}	83.32	15.83	17.34	38.14
Sarigol-1200	137.53^{c}	92.37	29.44	30.64	74.71	158.60^{b}	81.08	15.54	17.25	36.93
Mean	131.89	83.50	26.27	28.55	64.27	158.37	74.84	15.14	17.47	35.03
Number of seeds per fruit										
RGS003-0	21.63^{c}	76.97	9.93	11.31	22.99	21.55^{b}	74.63	9.70	11.23	22.13
RGS003-800	22.59^{b}	39.91	5.80	9.19	9.68	22.26^{a}	43.74	4.78	7.23	8.35
RGS003-1000	23.49^{a}	64.05	7.65	9.56	16.17	22.18^{a}	81.84	10.05	11.11	24.00
RGS003-1200	20.27^{d}	42.66	7.09	10.86	12.23	21.20^{b}	34.00	5.77	9.89	8.88
Sarigol-0	26.57^{a}	77.20	8.15	9.27	18.90	25.65^{a}	39.21	3.08	4.91	5.09
Sarigol-800	21.82^{b}	46.01	5.70	8.41	10.21	20.74^{c}	64.36	7.34	9.14	15.54
Sarigol-1000	21.44^{b}	46.67	6.27	9.18	11.31	22.53^{b}	56.19	6.27	8.37	12.42
Sarigol-1200	17.94^{c}	73.78	16.07	18.71	36.45	20.79^{c}	75.75	9.57	11.00	22.90
Mean	21.97	58.41	8.33	10.81	17.24	22.11	58.71	7.07	9.11	14.91
1000 seed weight										
RGS003-0	3.66^{a}	39.73	7.04	11.16	11.71	3.50^{a}	79.60	5.37	6.02	12.64
RGS003-800	2.62^{c}	47.55	15.33	22.22	27.90	3.08^{d}	70.33	12.19	14.54	26.99
RGS003-1000	2.56^{c}	82.59	22.30	24.54	53.51	3.23^{c}	90.51	12.21	12.83	30.66
RGS003-1200	2.97^{b}	52.21	14.70	20.35	28.05	3.32^{b}	80.26	11.66	13.01	27.57
Sarigol-0	3.51^{a}	86.98	9.53	10.22	23.47	3.30^{a}	24.92	3.27	6.55	4.31
Sarigol-800	2.79^{b}	89.63	29.83	31.51	74.56	3.26^{a}	89.16	13.75	14.56	34.27
Sarigol-1000	2.69^{b}	67.54	19.88	24.19	43.14	3.02^{b}	81.77	12.28	13.58	29.32
Sarigol-1200	2.36^{c}	66.84	20.99	25.68	45.31	2.61^{c}	72.62	12.07	14.16	27.15
Mean	2.89	66.63	17.45	21.23	38.46	3.16	73.65	10.35	11.91	24.11
Seed yield per plant										
RGS003-0	7.85^{c}	68.89	28.45	34.28	62.34	8.97^{d}	62.76	16.17	20.41	33.83
RGS003-800	8.83^{b}	63.98	29.72	37.16	62.76	11.62^{c}	47.70	13.54	19.61	24.69
RGS003-1000	10.23^{a}	88.21	34.61	36.85	85.81	14.62^{a}	94.31	29.41	30.28	75.40
RGS003-1200	5.50^{d}	73.31	30.64	35.78	69.25	11.93^{b}	85.39	20.31	21.98	49.54
Sarigol-0	6.83^{c}	86.65	34.53	37.09	84.85	7.89^{c}	42.87	13.71	20.94	23.70
Sarigol-800	9.67^{b}	86.53	36.41	39.15	89.43	9.59^{b}	76.79	19.55	22.31	45.23
Sarigol-1000	10.51^{a}	73.10	24.73	28.92	55.82	11.62^{a}	67.44	18.49	22.51	40.09
Sarigol-1200	7.14^{c}	75.06	28.69	33.12	65.63	9.69^{b}	69.88	16.06	19.21	35.45
Mean	8.32	76.97	30.97	35.29	71.99	10.74	68.39	18.40	22.16	40.99

[1]Means in each column with a common letter are not significantly differed at $LSD_{5\%}$.

Gamma rays increased the number of seed per fruit in M_2 and M_3 lines of RGS003 until 1000 Gy-treatment (Table 3). Mutation induction with higher dose (1200 Gy) reduced the number of seed per fruit in RGS003. These results are in agreement with those of Shah *et al.,* (1990) who isolated mutant line with higher number of seeds per fruit than control. In ‘Sarigol’, all mutagenic treatments reduced the values of this trait and control possessed the highest number of seed per fruit in both years. This result was consistent with that of Siddiqui *et al.*, (2009) in rapeseed and Patil *et al.,* (2004) in soybean who also observed a negative effect of mutagenic treatments on number of seeds per fruit. Gamma rays did not increase the heritability values in M_2 lines but the highest amount of this parameter were observed irradiating seeds with 1000 and 1200 Gy in M_3 lines of RGS003 and ‘Sarigol’ cultivars, respectively. Treatment with 1000 Gy gamma rays produced the highest genotypic coefficient of variation and genetic advance in M_3 lines of RGS003, while 1200 Gy-treatment showed the highest induced variability in M_2 and M_3 lines of ‘Sarigol’.

Irradiation mutagenic treatments negatively influenced 1000 seed weight in both generations (Table 3). The deleterious effect of the mutagen could be explained by the increased damage of cell constituents at molecular level or altered enzyme activity. The highest heritability estimate was obtained for 1000 Gy irradiation in RGS003 while 800 Gy-treated ‘Sarigol’ lines showed the highest heritability estimate in both generations. The coefficients of variation (phenotypic and genotypic) and expected genetic advance were increased in the treated populations as compared to control with the highest values belonging to M_2 lines of ‘Sarigol’ treated with 800 Gy. In both generations, mutagenic treatment with 1000 Gy of gamma rays was more effective in inducing the highest genetic variability in ‘RGS003’ while 800 Gy-treatment was the most effective one in ‘Sarigol’ cultivar.

Seed yield per plant was differently affected by gamma rays than its components increasing up to 1000 Gy and then decreases at 1200 Gy in both generations and cultivars (Table 3). Treatment with 1000 Gy dose induced the highest seed yield of M_2 and M_3 lines in both generations. The greater number of fruits per plant and seed per fruit, as two important yield components, produced at 1000 Gy-dose lead to the higher seed weight. The high yielding mutants has been developed from gamma rays irradiated population of rapeseed and mustard (Shah *et al.,* 1999; Siddiqui *et al.,* 2009). In both generations, 1000 Gy gamma rays caused the highest heritability, genotypic coefficient of variation and genetic advance in M_2 and M_3 lines of ‘RGS003’, while 800 Gy-treatment lead to the highest amount of these parameters in ‘Sarigol’ mutant lines.

In the present study, M_3 lines were more improved for all traits than M_2 lines. These improvements may be due to mutagenic-induced changes at the genetic level and selection of normal-looking plants in M2 which led to elimination of aberrant plants. Overall the highest genotypic and phenotypic coefficients of variation and genetic advance of means belonged to seed yield per plant followed by number of fruits per plant in both generations. The highest heritability estimated in the present study belonged to fruits per plant (83.5%) followed by seed yield (76.97%) in M_2 generation. On the other hand, plant height (79.96%) and days to maturity (77.9%) possessed the highest estimates of heritability in M_3 generation. These results indicate that higher broad-sense heritability does not necessarily lead to the higher genetic advance. Therefore, heritability per se is not an indication of genetic progress that can be achieved through selection (Larik *et al.,* 1999). However, when the estimates of heritability are used in combination with coefficient of variation and genetic advance parameters, a more realistic approach to select the traits can be acquired (Larik *et al*., 2009). This is in particular a consequence of involving non-additive genetic effects which in turn could weaken the selection efficiency for these quantitative traits (Larik & Rajput, 2000). .

Mutagen derived variability for quantitative traits in crop plants is heritable and its response to selection is reasonable (Frey, 1969). The results of the present study support this contention. Therefore, mutagenic-induced genetic variability can be successfully utilized to develop new cultivars of rapeseed with improved agronomic traits.

Correlation coefficients: Genotypic correlation coefficients between the traits in M_2 and M_3 were calculated and presented in Table 4. The results showed that seed yield was highly and positively ($p<0.01$) associated with all the traits with the exception of plant height (Table 4). Although seed yield (SY) positively correlated to days to flowering (DF) and days to maturity (DM) in M_2, their relationships were negative in M_3.

Number of fruits per plant (PP), number of seeds per fruit (SP) highly and positively correlated with SY in both generations (Table 4). Therefore, these yield components could be considered to be the most important contributors in seed yield improvement in canola. These results are in agreement with those of Diepenbrock (2000) and Karamzadeh *et al*., (2010) who also emphasized the contribution of these yield components in SY.

Plant height (PH) had a negative and significant correlation with SP in both generations. Belete (2011) in Ethiopian mustard and Karamzadeh *et al*., (2010) in canola have also reported a negative correlation between plant height and number of seeds per fruit. No significant relationship was found between PH and SY, and is consistent with that of Ozer & Oral (1999) and Tuncturk & Ciftci (2007) in canola.

The highest correlation coefficient in this study was observed between the phonological traits studied (DF and DM) in both M_2 and M_3 generations. PP correlated positively and significantly with DF and DM in M_2 generation. This relationship was also positive but not significant for M_3 generation. There was a positive, significant correlation between PP and SP in both generations. This relationship was also supported by Tuncturk & Ciftci (2007) in canola.

Path analysis: To determine yield-relating traits and yield components with direct and indirect effects, the data were subjected to path analysis. In both generations number of fruits per plant had the greatest positive effect on seed yield followed by SW and SP (Tables 5 & 6). A high direct effect of PP on yield with the justification values of 78% in M_2 and 61% in M_3 implies that this yield component is a reliable selection index to improve seed yield in canola. Although, in canola likewise the highest positive direct effect of PP on SY followed by SW and SP reported by Tuncturk and Ciftci (2007), no such discriminatory effect was observed for PP.

Negative indirect effect of PH via SP and SW could mask the positive direct effect of PH on SY and may lead to a negative correlation between PH and SY in both years (Tables 5 & 6). On the other hand, the high positive indirect effects of DM via DF and PP on SY were responsible for the positive relationship between DM and SY in M_2, even though the direct effect of DM on SY was negative and small.

The indirect effect of PP via SW was negative in both generations. This result indicated that higher PP could affect SY negatively by reducing SW, although high positive direct effect of PP on SY induced the highly significant and positive relationship between PP and SY (Table 4). This finding supports that of Ozer and Oral (1999) in spring rapeseed in which they observed a positive correlation between number of fruits per plant and seed yield despite the indirect effects of number of fruits per plant via number of seeds per fruit and 1000 seed weight.

Negative indirect effects of SW via PH, DF, PP and SP along with positive indirect effects of this trait through DM resulted in a positive genetic correlation between SW and SY in M_2 (Table 5). The high negative indirect effects of SW via PP was compensated with the positive direct effect of SW on SY (0.51) and resulted in highly significant and positive relationship between SW and SY. The negative indirect effect via PP is due to the compensatory effects in yield components and limitation in photosynthesis sinks in plant. Likewise, Sadat *et al.,* (2010) reported the negative indirect effect of 1000 seed weight on seed yield through number of fruits per plant. DF negatively correlated with SY (Table 4). The results of path analysis revealed the negative indirect effects through DM, SP and SW on SY could justify their inverse relationship.

Table 4. Genotypic correlation coefficients between studied traits in M_2 (upper of the diagonal line) and M_3 (lower of the diagonal line) generations of two cultivars of *Brassica napus* L.

Trait	PH	DF	DM	PP	SP	SW	SY
PH	1	0.08	-0.01	0.07	-0.28^{**}	-0.16^{**}	-0.07
DF	0.04	1	0.94^{**}	0.18^{**}	-0.18^{**}	-0.13^{**}	0.09^{*}
DM	-0.02	0.92^{**}	1	0.15^{**}	-0.16^{**}	-0.04	0.10^{*}
PP	0.01	0.12	0.11	1	0.21^{**}	-0.24^{**}	0.74^{**}
SP	-0.19^{**}	-0.01	-0.02	0.33^{**}	1	-0.03	0.45^{**}
SW	-0.09	-0.08	-0.12	-0.07	0.19^{**}	1	0.29^{**}
SY	-0.02	-0.22^{*}	-0.27^{*}	0.64^{**}	0.55^{**}	0.50^{**}	1

* and ** significant at 0.05 and 0.01 of probability levels, respectively
PH: plant height, DF: days to flowering, DM: Days to maturity; PP: number of fruits per plant, SP: number of seeds per fruit, SW: 1000-seed weight, SY: seed yield per plant

Table 5. Direct (diagonal and bolded) and indirect effects (upper and lower of the diagonal line) of morphophenological traits and yield components on seed yield in M_2 generation of two cultivars of *Brassica napus* L. using path coefficient analysis.

Trait	PH	DF	DM	PP	SP	SW
PH	**0.03**	0.01	0.001	0.05	-0.09	-0.08
DF	0.002	0.17	-0.10	0.14	-0.06	-0.07
DM	-0.001	0.16	-0.10	0.12	-0.05	0.02
PP	0.002	0.03	-0.02	0.78	0.07	-0.12
SP	-0.01	-0.03	0.02	0.16	0.32	-0.02
SW	-0.01	-0.02	0.004	-0.19	-0.01	0.51

PH: plant height, DF: days to flowering, DM: Days to maturity; PP: number of fruits per plant, SP: number of seeds per fruit, SW: 1000-seed weight, SY: seed yield per plant

Table 6. Direct (diagonal and bolded) and indirect effects (upper and lower of the diagonal line) of morphophenological traits and yield components on seed yield in M_3 generation of two cultivars of *Brassica napus* L. using path coefficient analysis.

Trait	PH	DF	DM	PP	SP	SW
PH	0.06	-0.001	0.005	0.006	-0.05	-0.04
DF	0.002	-0.02	-0.24	0.07	-0.003	-0.04
DM	-0.002	-0.02	-0.26	0.07	-0.01	-0.06
PP	0	-0.003	-0.03	0.61	0.09	-0.03
SP	-0.01	0	0.005	0.20	0.26	0.09
SW	-0.01	0.001	0.03	-0.04	0.05	0.47

PH: plant height, DF: days to flowering, DM: Days to maturity; PP: number of fruits per plant, SP: number of seeds per fruit, SW: 1000-seed weight, SY: seed yield per plant

Multiple stepwise regression analyses: Eighty three percent of the seed yield variation in M_2 generation could be explained by three seed yield component of number of fruits per plant (50%), 1000 seed weight (24%) and number of seeds per fruit (9%), while days to flowering and days to maturity could only justify 1% of the yield variation when entered into the regression model (Table 7). The positive and high regression coefficient between number of fruits per plant and seed yield further supports our above-mentioned findings of genotypic correlation coefficient and path analysis that this yield component is a reliable criterion for yield improvement in canola.

Number of fruits per plant, 1000 seed weight and number of seeds per fruit were the most important variables contributing seed yield in M_3 generation and totally explained 74% of the seed yield variation while days to maturity only justified 6% of the variation in M_3 as well. These yield components had a negative regression coefficient with days to maturity (Table 7). It appears that days to maturity negatively affects the seed yield in canola.

Table 7. Summary of stepwise multiple regression analyses of seed yield and related pheno-morphological traits in M_2 and M_3 generations of two cultivars of *Brassica napus* L. affected by gamma irradiation doses.

Regression equations	Coefficient of partial determination	Cumulative coefficient determination
M_2 generation		
SY=0.94+0.06PP	0.50	0.50
SY=-7.06+0.06PP+2.60SW	0.24	0.74
SY=-14.15+0.06PP+2.59SW+0.36SP	0.09	0.83
SY=-16.42+0.06PP+2.61SW+0.38SP+0.03DF	0.004	0.84
SY=-14.47+0.06PP+2.64SW+0.38SP+0.06DF-0.04DM	0.001	0.84
M_3 generation		
SY=1.85+0.06PP	0.38	0.38
SY=-9.57+0.06PP+3.53SW	0.28	0.66
SY=-17.25+0.05PP+3.20SW+0.46SP	0.08	0.74
SY=-7.56+0.05PP+3.03SW+0.45SP-0.07DM	0.06	0.80
SY=-9.12+0.05PP+3.06SW+0.47SP-0.07DM+0.01PH	0.004	0.81

PH: plant height, DF: days to flowering, DM: Days to maturity; PP: number of fruits per plant, SP: number of seeds per fruit, SW: 1000-seed weight, SY: seed yield per plant

Conclusions

In the present study we report a highly significant genotype × dose interaction for the agronomic traits in M_2 and M_3 which suggests that response to the gamma irradiation doses being genotype-dependent in terms of induction of genetic variations in canola. The results also showed the treated populations was significantly affected for all of the traits by the irradiations and the highest GCV, PCV and GAM belong to seed yield per plant. The highest variations induced with treatment of 1000 Gy of gamma rays in most of the traits in 'RGS003' cultivar, while 800 Gy gamma rays induced similar conditions in 'Sarigol' cultivar. The relationship between traits based on correlation coefficients, path analysis and multiple regression analyses revealed major contribution of number of fruits per plant on justifying seed yield variation in both M_2 and M_3 generations. These results indicate that this yield component is a reliable criterion for yield improvement in canola.

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