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Molecular and morphological assessment of genetic variability induced by gamma radiation in canola

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Abstract
Mutation induction is considered as an effective way to enrich plant genetic variation, particularly for traits with a very low level of genetic variation. This research was conducted to assess genetic variation induced by gamma radiation in M_2 and M_3 mutant lines of canola (*Brassica napus* L.) by SSR and morphological characteristics and to identify useful mutants in terms of agronomic traits. Sixty-two mutant lines derived from gamma mutagenesis and their wild-type progenitors ('RGS003' and 'Sarigol' cvs) were used. Twenty-five polymorphic SSR primers were used in this study. Results of cluster analysis based on both morphological traits comprising plant height, days to flowering, days to maturity, number of pods/plant, number of seeds/pod, 1000-seed weight and seed yield/plant and SSR data revealed a separate grouping of mutant lines from control cultivars. SSR data analysis of mutant lines and controls demonstrated a considerable genetic variation among mutant lines, where 83% of primers generated polymorphic bands with 3.32 alleles per locus. The genetic distance calculated between mutant lines and their controls indicated a significant difference between mutant lines and controls. Although both morphological and SSR markers successfully discriminated mutant lines from controls, SSR primers could further discriminate between the mutant lines derived from the related cultivar. Mutant lines 24 derived from 'RGS003' and 16 and 26 from 'Sarigol' were considered as superior for breeding canola, which could be utilized in future genetic and breeding programs. Distinct classification of genotypes based on agro-morphological and SSR data in the present study implies that morphological and SSR markers reflected different aspects of genetic variation among mutant lines.

***Key words*:** canola, gamma rays, genetic diversity, mutation, SSR.

Introduction

Canola (*Brassica napus* L.) is the most important source of edible oil and the second most important oilseed crop in the international oilseed market after soybean (Hasan *et al.* 2006). The interest of canola production has increased significantly worldwide in the last few decades. Canola has been widely grown in Iran in recent years for oil production and rarely livestock feed. High seed yield (over two tones per hectare) and high oil content (approximately 40%), in addition to low water needs in dry regions of Iran, have made it one of the most important crop plants that provide the oil needs of the country. Due to the

economic importance of oilseed rape, several *B. napus* cultivars from diverse sources in the world have been introduced in Iran during the last decade and at present. Canola cultivars appear to be best adapted to the conditions of Iran. However, some cultivars are less tolerant to environmental conditions (Shargh *et al*. 2011). Extensive genetic uniformity of Canola cultivars can lead to crop losses if there is susceptibility to the spread of disease or pest infestations or to severe environmental stress (Hajjar *et al*. 2008). Availability of genetic variability is the prerequisite for any breeding program. Besides conventional methods, induced mutation has been extensively used for creating new genetic variation in crop plants (Parry *et al*. 2009).

To date, 198 mutant cultivars of annual oilseed crops including soybean, sesame, canola, sunflower and linseed have been released (FAO/IAEA, 2012). Mutation induction with radiation was the most commonly used method to develop direct mutant cultivars. During the period 1930–2004 gamma rays were used to develop 64% of the radiation-induced mutant cultivars (Ahloowalia *et al.* 2004).

Induced genetic variability can be assessed at morphological and molecular levels. During last decades, approaches based on DNA markers have been used to detect variation at DNA level and to determine interrelationships among closely related genotypes (Alan, 2007). A variety of molecular markers including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorfic DNA (RAPD), anchored simple sequence repeat polymorphism (ISSR) and simple sequence repeats (SSR) have been used to estimate genetic diversity among the diverse group of important crops in the genus *Brassica* (Halldén *et al*. 1994; Charters *et al*. 1996; Negi *et al*. 2001; Riaz *et al*., 2001; Hasan *et al*., 2006). RFLP analysis is renowned for its reliability and codominant nature but is time-consuming, relatively expensive and requires considerable technical expertise. AFLP analysis is fast, highly polymorphic and reproducible. However problems of dominant nature, need for purified, high molecular weight DNA, and the possible non-homology of comigrating fragments belonging to different loci have been noted.

ISSR technique is very simple, highly discriminative and reliable. However problems of dominant nature, low reproducibility and nonhomology of similar sized fragments have been noted (Jaroslava *et al*. 2002; Jiang *et al*. 2007).

Simple sequence repeats (SSRs) also known as microsatellite markers are preferred by many geneticists and plant breeders because of their properties of genetic co-dominance, abundance, dispersal throughout the genome, multiallelic variation, high repeatability and amenability to high throughput automated allele detection and sizing (Plieske and Struss, 2001; powell *et al*. 2006).

Microsatellite markers may provide an efficient method to characterize artificially induced genetic variability in crop plants. Microsatellite instability characterized by slippage-induced mutation

and defect in mismatch repair in simple sequence repeats make this marker as excellent system for the efficient monitoring of spontaneous and induced mutations in higher plants and animals (Vigouroux *et al*. 2002; Kashi and King, 2006). The length, i.e. copy (or repeat) number, and point mutations lead to the allelic variants and the possible evolutionary dynamics of microsatellites, respectively (Bhargava and Fuentes, 2010).

A number of studies have been devoted to estimating the relative rates of spontaneous mutation in microsatellite loci in plants (Vigouroux *et al*. 2002; Marriage *et al*. 2009).

Gamma-radiation is an ionizing radiation known to cause a wide range of intragenic and intergenic mutational changes. The range of mutation events caused by ionizing radiation varies from simple base substitutions to single and double strand breaks of DNA molecule. The changes in the structure and function of the genome coupled with an increase in mutation rates are part of the complex response of an organism to exposure to ionizing radiation (Kovalchuk *et al*. 2000; Esnault *et al*. 2010; Kuchma *et al*. 2011;).

Canola is a recent introduced crop to Iran and has major cultivation problems such as seed shattering and aphid susceptibility. Therefore, the present study is a part of mutagenesis program aiming at induction of genetic variation for the desirable agronomic traits. The particular objectives of this study were mainly to determine the effect of gamma radiation on genetic variability of mutant plants and their classification using SSRs and morphological markers.

Materials and Methods

Plant materials

In the present study, 62 canola genotypes comprising 32 mutant lines derived from 'RGS003', 30 mutant lines derived from 'Sarigol' along with their parent cultivars were used. 'RGS003' was originated from Germany while 'Sarigol' is an Iranian cultivar. Seeds were treated with 800, 1000 and 1200 Gy gamma irradiation and M_1 plants were evaluated under field conditions. Selected M_1 plants for their superiority in their maturity date, aphid infestation, and seed shattering properties were then harvested. M_2 seeds of the selected M_1 lines were used in this study for assessing morphological variations. The selected plants were bagged at flowering to prevent cross-pollination.

Field experiment

The experiment was carried out at the research farm of Isfahan University of Technology, Iran (32^o 32' N and 51^o 32' E) using a randomized complete block design with 5 replications. Sixty-two M_2 mutant lines along with the parent controls were evaluated in the field during two growing seasons of 2010-2011. Days to flowering, days to maturity, plant height (cm), number of pods per plant, number of seeds per pod, 1000 seed weight (g) and seed yield per plant (g) were recorded using five randomly selected plants from each line. Clustering of mutant lines along with controls was performed using un-weighted pair group method with arithmetic mean

(UPGMA) and Euclidean distance coefficient by NTSYS-pc software v2.2 (Rohlf, 2008). The appropriate number of groups was defined according to the *pseudo-F* and *pseudo-t*^{2} criteria (SAS Institute, 2011). Means of mutant lines were compared with those of controls, using Fisher's least significant difference (LSD) procedure.

DNA isolation and PCR amplification

Leaf samples were taken from 10 bulked plants in each M_2 mutant line and/or control plants. DNA was extracted according to CTAB protocol (Murray and Thompson, 1998) with minor modification. Leaf tissues from each sample were cut into small pieces and homogenized and digested with extraction buffer (CTAB 2%, 100 mM Tris, 1.4 M NaC1, 20 mM EDTA, pH 8.0). After incubation for 30 minutes at 65 ^{oC} with intermittent swirling, DNA was isolated with chloroform: isoamyl alcohol mixes (24:1) and precipitated using two volume of absolute ethanol in presence of 0.3M sodium acetate and pelleted by centrifugation. The pellets were then washed with 70% ethanol, air dried and dissolved in distilled water.

A PCR reactions were carried out in a volume of 20 l containing 30 ng of template DNA, 4 pmol of each primer, 3mM each dNTPs, 1 unit of *Taq* DNA polymerase, 2.5 mM $MgCl_2$ and 1x PCR buffer.

Out of 404 *Brassica* microsatellite primers, a total of 30 microsatellite primer pairs selected from the publicly-available collection of *Brassica* SSRs (www.brassica.info/ssr/ SSRinfo.htm) were used. The amplification reactions were carried out using the following thermal profile: 95 $^{\circ}$C for 5 min, 35 cycles of 30 s at 94 $^{\circ}$C, 60 s at the specific-primer annealing temperature (Table 1), and 60 s at 72 $^{\circ}$C. The last cycle was followed by 10 min at 72 $^{\circ}$C, and the PCR reaction was terminated with a continuous cycle at 4 $^{\circ}$C. The amplification reactions were performed using a DNA thermal cycler (Bio-Rad, Model 580BR). The amplification products were separated by electrophoresis on 12% non-denaturing polyacrylamide gels (Atto, Tokyo, Japan) and visualized by silver staining with a 50 bp DNA ladder (Fermentas, Germany) as reference.

Table 1. PCR amplification program used in this study.

Segment	Purpose	Time	Temperature
1	Initial denaturation	5 min	95 $^{\circ C}$
35	Denaturation	30 sec	94 $^{\circ C}$
	Annealing	1 min	* $^{\circ C}$
	Extension	1 min	72 $^{\circ C}$
1	Final extension	10 min	72 $^{\circ C}$

*Annealing temperature depends upon primer type as presented in Table 4

Data analysis

The DNA fragments amplified by microsatellite primers were scored as present (1) or absent (0) and recorded with reference to the molecular weight markers. The data were then analyzed as if they were dominant markers, since it was not possible to calculate gene frequencies as a result of bulking 10 plants per M_2 line. Number of polymorphic bands, percentage of polymorphism and polymorphic information content (PIC) were calculated for each primer combination. UPGMA dendro-

gram were constructed based on Dice similarity coefficient (Nei and Li, 1979) using NTSYS software version 2.2 .The non-parametric analysis of molecular variance (AMOVA) described by Excoffier *et al*. (1992) was performed to determine a cut point in the dendrogram and to partition the variation into within mutant lines, between two groups of mutant lines and between mutant lines and controls using Arlequin software v3.1. The cophenetic correlation coefficient between molecular and morphological data was calculated using the Mantel test based on their similarity matrices and related dendrogram derived matrices, respectively.

Results

In this study only lines derived from the highest dose (1200 Gy) radiation having the greatest seed mortality were used for morphological and molecular analysis.

Morphological traits

A cluster analysis based on the phenotypic distance matrix (Figure 1) revealed at least five major groups (M1-M5). Mean comparisons of the five groups for various traits indicated that there is a significant difference between the groups for number of pods per plant (Table 2). Mutant line number 26 with 'Sarigol' genetic background was clustered into a separate group. This line produced the highest number of pods per plant, 1000 seed weight and seed yield per plant. On the other hand, group 2 comprised 6 mutant lines possessing the lowest number of pods per plant, number of seeds per pod, 1000 seed weight and seed yield per plant.

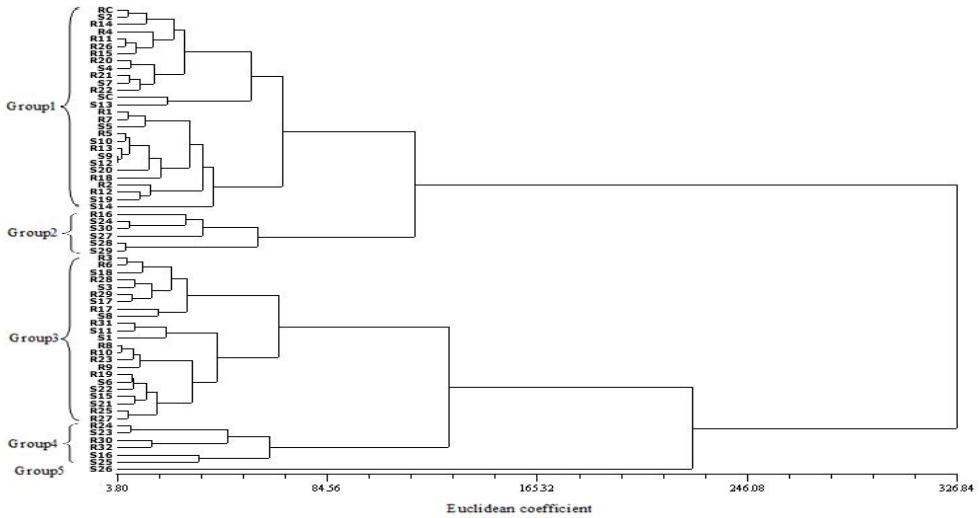

Figure 1. Dendrograms of 62 mutant lines and two controls ('RGS003' and 'Sarigol') of canola genotypes obtained by UPGMA cluster analysis based on morphological traits (S1-S30= mutant lines of 'Sarigol', R1-R32= mutant lines of 'RGS003', SC and RC= 'Sarigol' and 'RGS003' cultivars, respectively).

Table 2. Means of agronomic traits for each group derived from cluster analysis of 62 mutant lines along with two controls (RGS003 and 'Sarigol').

Group	Days to flowering	Days to maturity	Plant height (cm)	Number of pods/plant	Number of seeds/pods	1000 Seed weight (g)	Seed yield/ plant (g)
1	68.13 [b*]	115.59 [b]	73.29 [a]	78.41 [d]	13.57 [a]	3.28 [c]	8.82 [b]
2	89.38 [a]	126.33 [a]	53.83 [b]	10.00 [e]	3.03 [b]	1.05 [d]	3.12 [c]
3	67.01 [b]	115.13 [b]	74.08 [a]	132.31 [c]	15.59 [a]	3.57 [bc]	9.63 [b]
4	78.00 [ab]	120.00 [ab]	63.26 [ab]	206.72 [b]	16.07 [a]	4.32 [ab]	10.45 [ab]
5	78.00 [ab]	124.00 [a]	77.32 [a]	325.00 [a]	13.80 [a]	4.90 [a]	11.77 [a]

*Means followed by the same letter are not significantly different at $LSD_{5\%}$.

Cluster analysis using means of morphological traits divided mutant lines and controls into the separate groups whereas the mutant lines belonging to two genetic backgrounds were not clearly divided (Figure 2). Significant differences were observed between mutant lines and related controls for the traits under evaluation (Table 3). Mutant lines were shorter in stature than the controls. On average, mutant lines matured earlier than their parental cultivars. Reduction in the plant height and maturity was compensated with increase in both numbers of pods per plant and seed weight in the mutant lines.

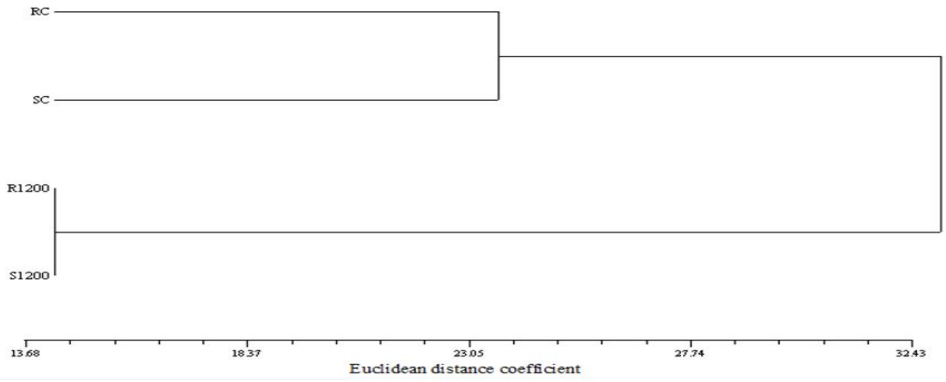

Figure 2. UPGMA dendrogram of 62 mutant lines and two controls ('RGS003' and 'Sarigol') based on means of morphological traits (S1200 and R1200 = means of mutant lines of 'Sarigol' and 'RGS003', respectively, SC and RC= 'Sarigol' and 'RGS003' cultivars, respectively).

Table 3. Mean comparisons of agronomic traits in mutant lines (average of M_2 and M_3) and controls.

Group	Days to flowering	Days to maturity	Plant height (cm)	Number of pods/plant	Number of seeds/pods	1000 seed weight(g)	Seed yield /plant (g)
Control	76.8 [a*]	122.30 [a]	85.66 [a]	85.14 [b]	21.93 [a]	3.24 [a]	7.75 [b]
Mutant lines	70.78 [b]	116.90 [b]	71.21 [b]	110.58 [a]	13.46 [b]	3.47 [b]	8.96 [a]

*Means followed by the same letter are not significantly different at $LSD_{5\%}$.

Microsatellite loci

Genetic variability

Evaluation of genetic variability at the DNA level in mutant lines and their progenitors (controls) was also conducted. Among 30 SSR primer pairs, 25 were polymorphic and showed variation among mutant lines. Out of the polymorphic primers, 24 and 22 generated polymorphic bands among the mutant lines and related controls, 'Sarigol' and 'RGS003', respectively.The total number of scoreable loci were 83, out of which 77% were polymorphic and 23% were monomorphic (Table 4). Of the 83 alleles detected 4 alleles (4.82%) were considered rare. The number of alleles produced by various primers ranged from 1 to 6, with an average of 3.32 alleles per locus. The maximum number of alleles was detected at NA10-D11, O110-B06 and BN12A SSR loci (6 alleles). The discrimination power of each SSR locus was estimated by the PIC. PIC values ranged from 0.08 for locus NA10-C06 to 0.54 for locus NA10-G08 and O110-H07 with a mean of 0.43 (Table 4, Figure 3).

Table 4. SSR primers, number of fragments percentage of polymorphism, polymorphism information content (PIC) and gene diversity in 62 mutant lines and two parents (control).

Primer	Repeat motif	Annealing temp	Total number of alleles	Number of Polymorphic alleles	Percentage of polymorphism (%)	PIC	Gene diversity
NA10-D11	$(GA)_{27}$	61	6	6	100	0.31	0.38
NA10-G08	$(CT)_{16}$	58	3	3	100	0.54	0.47
O110-B06	$(GA)_{21}$	61	6	6	100	0.41	0.49
O110-H07	$(GA)_{29}$	63	5	3	60	0.54	0.37
RA2-E12	$(GA)_{32}$	59	2	1	50	0.08	0.032
NA10-C06	$(GA)_{58}$	56	2	1	50	0.51	0.45
NA12-D04	$(CA)_{11}$	60	3	2	66.6	0.51	0.50
NA12-E05	$(CA)_{10}$	58	3	1	33.3	0.50	0.57
NA12-F03	$(GA)_{35}$	60	5	5	100	0.48	0.43
NA12-H02	$(GA)_{12}$	59	4	2	50	0.41	0.50
NA14-H12	$(AC)_{16}$	56	2	1	50	0.41	0.46
NI2-F02	$(CT)_{29}$	56	2	2	100	0.40	0.47
0113-E08	$(CT)_{11}$	61	4	1	25	0.39	0.43
BN12A	$(GA)_{11}(AAG)_4$	66	6	6	100	0.43	0.36
NA12-A02	$(CT)_{16}$	57	4	4	100	0.44	0.35
NA12-D10	$(CT)_{14}$	57	3	1	33.3	0.49	0.47
NA14-F11	$(GT)_7$	60	3	3	100	0.45	0.46
NI4-D09	$(CT)_{25}$	57	2	1	50	0.45	0.25
O110-B04	$(CT\text{-}CA)_{9\text{-}11}$	60	2	2	100	0.47	0.36
O110-F07	$(CT)_{15}$	56	2	2	100	0.47	0.45
O112-F11	$(AC)_{14}$	61	2	2	100	0.53	0.42
O113-H09	$(GT)_{10}$	56	3	1	33.3	0.44	0.43
RA2-E11	$(CT)_{24}$	62	3	3	100	0.21	0.36
NA10-B10	$(GGC/A/T)_{18}$	59	2	2	100	0.51	0.48
NA12-A08	$(GA)_{28}$	60	4	3	66.6	0.51	0.45
Mean			3.32	2.56	74.7	0.43	0.42

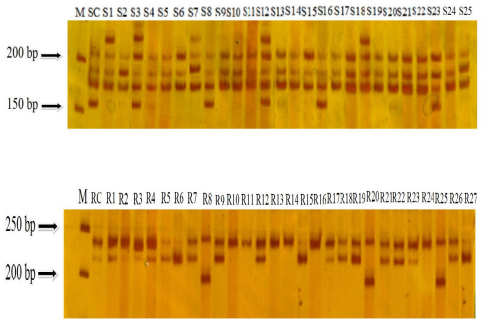

Figure 3. PCR amplification profile of canola 0110-H07 and NA10-G08 SSR markers in M_2 mutant lines derived from 'Sarigol' and 'RGS003' cultivars respectively. (S1-S25= mutant lines of 'Sarigol', R1-R27= mutant lines of 'RGS003', SC and RC= 'Sarigol' and 'RGS003' cultivars, respectively), Lane M: 50 bp DNA ladder.

The gene diversity of microsatellite markers ranged from 0.032 (primer NA10-C06) to 0.57 (primer NA12-E05) with an average of0.40.

The allelic variation of mutant lines with 'Sarigol' background was relatively high, ranging from 19.0% to 56.5% and 35.3% and was greater than of the control (Table 5). Moreover, the allelic variation of mutant lines belonging to 'RGS003' cultivar ranging from 25.4% to 53.1% and was 40.4% greater than control (Table 5).

Cluster analysis

The dendrogram generated using the Dice similarity coefficients and based on 24 SSR loci, which were common in Sarigol populations, grouped the 31 genotypes into 6 clusters (Figure 4). The first group included 8 mutant lines along with their progenitor. Group 2 included 4 mutant lines. Based on morphological traits, mutant lines number 3, 9, 12 and 16 of this group possessed the highest 1000 seed weight. Group 3 comprised 10 mutant lines. Group 4 had 2 mutant lines. Mutant lines number 8 and 11 of this group possessed the highest number of pods per plant, number of seeds per pod and seed yield per plant.

Table 5. Changing of SSR alleles between mutant lines and parents of 'Sarigol' and 'RGS003' cultivars. S1-S30= mutant lines of 'Sarigol', R1-R32= mutant lines of 'RGS003'.

Mutant lines	(%)*	Mutant lines	(%)*
S1	27	R1	31
S2	24	R2	31
S3	35	R3	32.72
S4	24	R4	34.72
S5	37	R5	36.40
S6	25.40	R6	32.72
S7	29.40	R7	34.54
S8	48.60	R8	36.40
S9	31.74	R9	34.54
S10	44	R10	43.63
S11	44.60	R11	38.20
S12	47	R12	29.09
S13	43.80	R13	29.09
S14	56.50	R14	34.54
S15	38	R15	32.72
S16	44.30	R16	38.20
S17	36.50	R17	47.30
S18	27	R18	36.50
S19	30.16	R19	40
S20	47	R20	31
S21	24	R21	43.63
S22	19.04	R22	40
S23	31.74	R23	33
S24	25.40	R24	53.09
S25	30.16	R25	25.45
S26	31.74	R26	32.16
S27	49.62	R27	31.74
S28	38.09	R28	41.82
S29	27	R29	49.82
S30	41.30	R30	47.45
		R31	34.54
		R32	40
Mean	35.30		40.40

*: (%) different alleles between mutant lines and parents.

Group 5 included 4 mutant lines and Group 6 had 2 mutant lines.

The dendrogram generated using the Dice similarity coefficients and based on 24 SSR loci, which were common in 'RGS003'populations, grouped the 33 genotypes into 5 clusters (Figure 5). The first group included 9 mutant lines along with their progenitor. Group 2 included 14 mutant lines. Group 3 comprised 7 mutant lines. Mutant lines number 9, 16, 19, 21, 22, 23 and 26 of this group possessed the highest number of seeds per pod and seed yield per plant. Group 4 had only one mutant line. Mutant line number 29 of this group possessed the highest 1000 seed weight and Group 5 had 2 mutant lines.

The dendrogram generated using the Dice similarity coefficients and based on 22 SSR loci, which were common in both mutant populations, grouped the 64 genotypes into 7 clusters (Figure 6). All mutant lines belonging to 'RGS003', with the exception of one, along with their progenitor were clustered in group 1. Group 2 comprised only a mutant line (number 24) with 'RGS003' background. Mutant lines of 'Sarigol' were clustered in group 3 to group 6 group 6 included only one mutant line (number 16). Based on morphological traits, mutant line number 24 of 'RGS003' possessed the highest 1000 seed weight, while mutant line number 16 of 'Sarigol' was a superior line for plant stature (shortest height), number of pods per plant, number of seeds per pod and seed yield per plant.

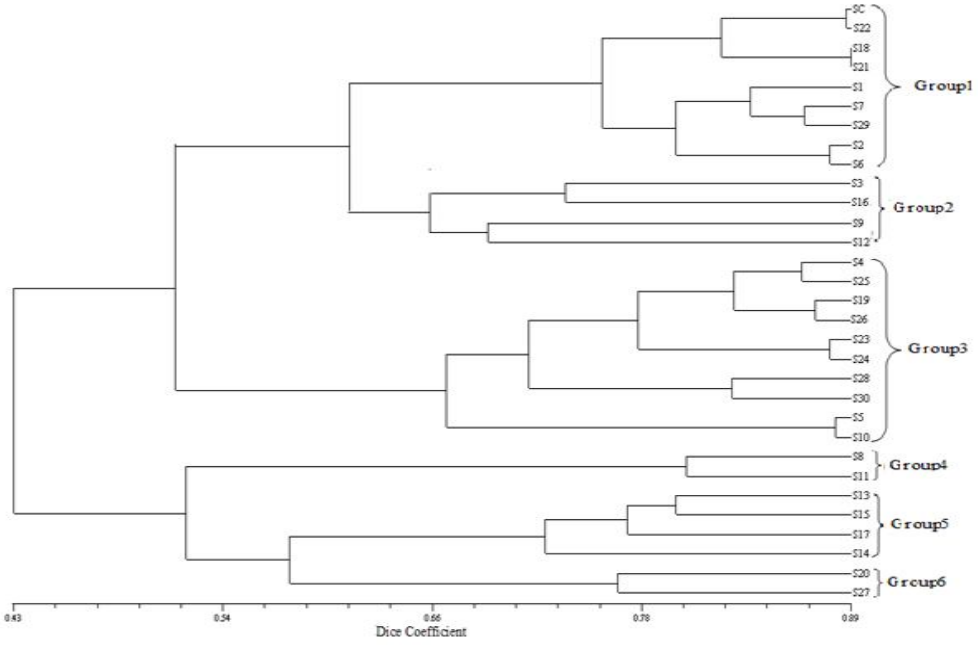

Figure 4. UPGMA dendrogram of 30 mutant lines and control parent of 'Sarigol' based on SSR markers (S1-S30= mutant lines of 'Sarigol', SC = 'Sarigol' cultivar).

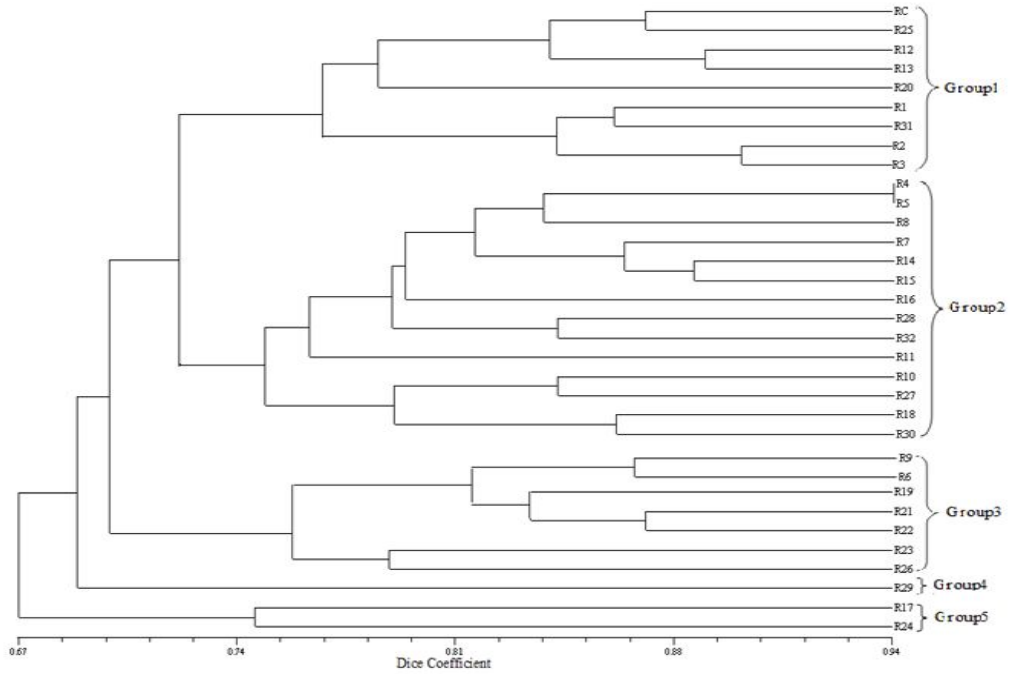

Figure 5. UPGMA dendrogram of 32 mutant lines and control parent of 'RGS003' based on SSR markers (R1-R32= mutant lines of 'RGS003', RC = 'RGS003' cultivar).

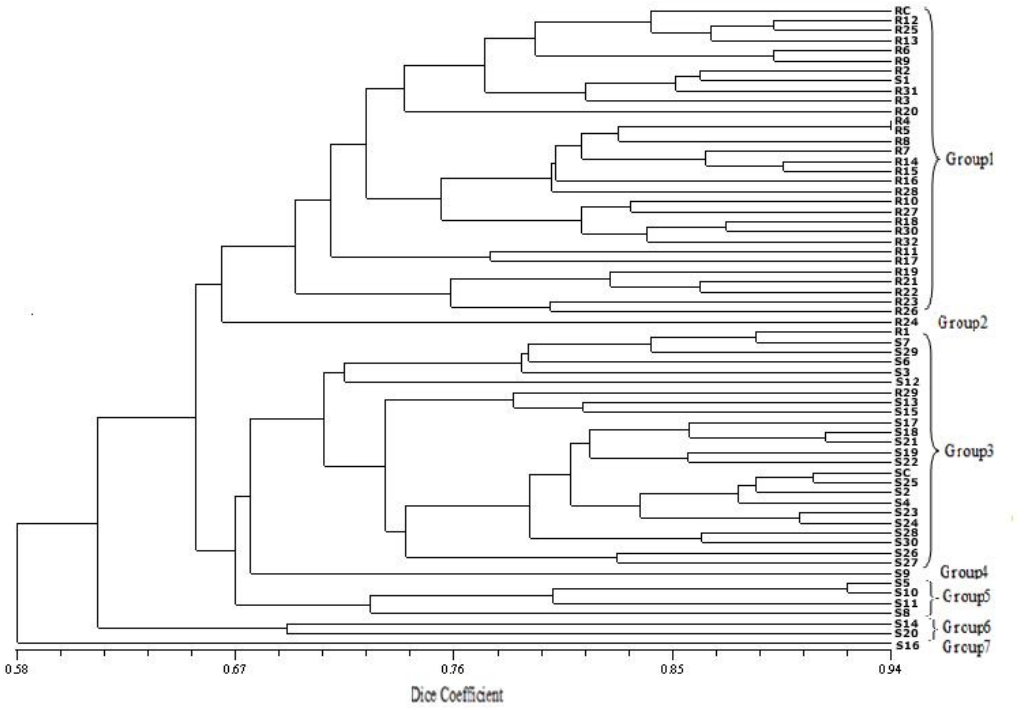

Figure 6. UPGMA dendrogram of 62 mutant lines and two control of 'RGS003' and 'Sarigol' based on SSR markers (S1-S30= mutant lines of 'Sarigol', R1-R32= mutant lines of 'RGS003', SC and RC= 'Sarigol' and 'RGS003' cultivars, respectively).

The AMOVA results revealed a significant difference between two groups of the mutant lines (Table 6). A significant fixation index value, FST = 0.216, observed in the present study further confirmed the distinction between these two groups. Furthermore; a highly significant variation was observed within each of the two groups. Results of AMOVA indicated a significant difference between mutant lines and controls (Table 7).

Comparison between the clusters generated based on morphological traits and SSR markers indicated a small cophenetic correlation coefficient (r = 0.18).

Table 6. Results of analysis of molecular variance (AMOVA) between two groups (32 mutant lines of 'RGS003' vs. 30 mutant lines of 'Sarigol') and the calculated fixation index (F_{ST}*).

Source of variation	df	Sum of squares	Variance components	Percentage of variation	P
Between two groups of mutant lines	1	71.93	2.08	21.61	<0.000001
Within mutant lines	60	452.6	7.54	78.39	
Total	61	524.5	9.62		

*Fixation index F_{ST}=0.216, significant tests over 1000 permutations.

Table 7. Results of analysis of molecular variance (AMOVA) between two groups of 62 mutant lines vs. 2 cultivars'RGS003' and 'Sarigol' (controls) and the calculated fixation index (F_{ST}*).

Source of variation	df	Sum of squares	Variance com-poents	Percentage Of variation	P
Mutant lines vs controls	1	5.49	0.61	12.50	<0.000001
Within mutant lines	62	264.7	4.27	87.50	
Total	63	270.2	4.88		

*Fixation index F_{ST}=0.125, significant tests over 1000 permutations.

Discussion

The experiments were designed to test the hypothesis that the maximum tolerable gamma irradiation (1200 Gy) can enhance diversity at both morphological and microsatellite levels in canola. Cluster analysis using means of 7 morphological traits divided mutant lines and controls into the separate groups. The differences between the two groups were statistically significant. It can be inferred from the results that gamma radiation has induced significant genetic variability in canola with regard to morphological traits. Compared to important cereal and oilseed crops, limited research on mutation breeding has been conducted in canola to enhance the genetic variability for agronomic and morphological traits. On the other hand, oil quality, particularly the fatty acid profiles, has been modified in *Brassica* species using natural and artificial mutations (McVetty and Scarth, 2002;

Wittkop *et al*. 2009). Some agronomically desirable mutant lines detected in the present study could reinforce the successful implementation of performance change in canola via mutation breeding. Induction of mutations for seed yield and yield component improvement in oilseed *Brassica* has also been reported by other researchers (Shah *et al*. 2005; Kumar *et al*. 2011).

In the present study, different microsatellite haplotypes observed among mutant lines having a similar genetic background are most likely induced by treatment with the physical mutagen (gamma rays at 1200 Gy). Moreover, analysis of the SSR banding patterns showed a significant difference between the mutant lines and the control parents. Although SSRs have been extensively used in studies encompassing various areas of genetics, the mutation dynamics of these genomic regions is still not well elucidated. Repetitive or highly repeated regions such as microsatellites are usually hypervariable sequences. Single-strand slippage replication effects and defect in mismatch repair have been proposed as the main mutational mechanisms accounting for the high mutation rates observed in microsatellite loci (Vigouroux *et al*. 2002). In addition, genomic constitution of the flanking sequences can greatly associates to the mutability of microsatellites. Large scale mutations of a sequence that contains or brackets a microsatellite modify the genomic context of the microsatellite and may change the mutability of the locus (Buschiazzo and Gemmell, 2006).

The rate of SSR mutation also depends on motif length, motif sequence, number of repeats and repetition purity (Vigouroux *et al*. 2002; Symonds and Lioyd, 2003; Kashi and King, 2006). The repeat purity can be degraded by point mutation and thereby stabilize an SSR, whereas the imperfect repeats may be eliminated by active mutational slippage (Kashi and King, 2006). In the present study, most of the SSRs have di-nucleotide repeat motifs (92%) and majority of which (72%) had more than 11 repeat number. This result is in agreement with that of recent studies showing that slippage rates are higher in di-nucleotide repeats, followed by tri- and tetra-nucleotide repeats (see review by Bhargava and Fuentes, 2010) , and also SSRs with longer repeat numbers generate more mutated alleles than shorter ones (Marriage *et al*. 2009).

The results of cluster analysis and AMOVA based on SSR data also confirmed a considerable genetic variation induced by gamma rays in the mutant lines, between the mutant lines belonging to two different genetic backgrounds and between the mutant lines and the controls. Hence, variability induced by gamma radiation is likely to be genotype- dependent. When open-pollinated seeds are used, besides the radiation induced mutation, segregation due to the residual heterozygosity and out-crossing of M_1 plants may also account for the observed variation. However, this may not be relevant to the present study since M_1 plants were bagged at the flowering stage to prevent cross contamination and 10 pooled M_2 plants from each of the lines and con-

trols were used to isolate DNA to weight the residual heterozygosity between the mutant lines and controls. Still, it has to be acknowledged that part of genetic diversity observed in the present study may be accounted by severe selection made in M_1 plants. Our observation of high mutation rate of SSR loci in canola are in agreement with the observation in barley by Mlcochova *et al*. (2004), but contradict with that of Fu *et al*. (2008) in rice.

This study demonstrate that SSR markers were more efficient compared to the morphological traits in discriminating mutant lines having different genetic backgrounds. Therefore, SSR markers can be useful in assessing genetic diversity induced by mutation. This result is in agreement with that of Kumar *et al*. (2011) who reached to the same conclusion using ISSR markers. Nevertheless, distinct classification of genotypes based on agro-morphological and SSR data in the present study implies that morphological and SSR markers reflect different aspects of genetic variation among mutant lines.

Conclusions

To the best of our knowledge, this is one of the first studies to reveal the association between agro-morphological traits and SSR markers for genetic variability induced by gamma radiation in canola. The current study showed that 1200 Gy gamma radiation can induce significant genetic diversity at both morphological and molecular levels that can be exploiting in the canola breeding programs. The shorter stature and, on average, earlier maturity mutant lines in comparison with their parental controls along with some of the lines that exhibited superior agronomic performance further support the significant role of gamma rays in the alteration of plant architecture on which selection can operate.

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بررسی تنوع مولکولی و مورفولوژیک القا شده با پرتوتابی گاما در کلزا

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چکیده

القای جهش راهکاری موثر در جهت افزایش تنوع ژنتیکی گیاهان، بویژه برای صفاتی با سطوح پائین تنوع ژنتیکی می‌باشد. این مطالعه به منظور بررسی تنوع ریزماهواره‌ای و مورفولوژیک القا شده با اشعه گاما در لاین‌های M_2 و M_3 کلزا (Brassica napus L.) و شناسایی موتانت های مفید از لحاظ صفات زراعی اجرا شد. شصت و دو لاین موتانت حاصل از جهش‌زایی با گاما به همراه ارقام شاهد تیپ وحشی (ارقام RGS003 و Sarigol) استفاده شد. بیست و پنج آغازگر چند شکل SSR هم در این مطالعه بکار رفت. نتایج تجزیه خوشه‌ای بر اساس هر دو داده‌های مورفولوژیک شامل صفات ارتفاع بوته، روز تا گلدهی، روز تا رسیدگی، تعداد غلاف در بوته، تعداد دانه در غلاف، وزن هزار دانه و عملکرد دانه در بوته و SSR نشان داد که لاین‌های موتانت از ارقام شاهد تفکیک شده‌اند. ضمن اینکه تجزیه و تحلیل داده‌های SSR نشان داد که تنوع ژنتیکی قابل ملاحظه‌ای میان لاین‌های موتانت القا شده، بطوری که ۸۳٪ آغازگرها باندهای چندشکل با ۳/۳۲ آلل در هر مکان ژنی ایجاد کردند. فاصله ژنتیکی محاسبه شده بین لاین‌های موتانت و ارقام شاهد حاکی از اختلاف معنی‌دار بین لاینهای موتانت و شاهد بوده است. مشابها، تجزیه و تحلیل واریانس مولکولی اختلاف معنی‌داری بین لاینهای موتانت و شاهد را نشان داد. اگرچه هر دو نشانگرهای مولکولی و مورفولوژیک بطور موفقی لاین‌های مونانت را از شاهد تفکیک کردند، اما نشانگرهای SSR لاین‌های حاصل از دو رقم را هم از یکدیگر جدا کرد. با در نظر گرفتن تنوع ژنتیکی القا شده سودمند برای برنامه های اصلاحی، لاین‌های موتانت ۲۴ RGS003 و ۱۶ و ۲۶ متعلق به Sarigol برای اصلاح کلزا برتر بوده و قابل استفاده در برنامه‌های ژنتیکی اصلاحی آینده کلزا می باشند. وجه تمایز گروه‌بندی ژنوتیپ‌ها بر اساس نشانگرهای مورفولوژیک و SSR حاکی از جنبه‌های متفاوت تنوع ژنتیکی القا شده در لاین‌های موتانت بوده است.

کلمات کلیدی: کلزا، اشعه گاما، تنوع ژنتیکی، جهش، ریزماهواره.