EFFECT OF GAMMA IRRADIATION ON MORPHOLOGICAL, PHYSIOLOGICAL AND MOLECULAR TRAITS OF *Brassica napus*

A. A. M. YASSEIN[1] AND AMINA A. ALY[2]

1. *Genetics Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt*
2. *National Centre for Radiation Research and Technology, Nasr City, Cairo, Egypt*

Brassica napus L., (2n =AACC = 38) is an amphidiploid, originated through natural crossing between *B. oleracea* (2n = AA =18) and *B. nigra* (2n = AA = 20) (Larik and Hussain, 1990). Rapeseed is one of the most important oilseed crops and considered as the most promising oil crops characterized with high seed oil content (40-45%). Cultivation of rapeseed in Egypt may provide an opportunity to overcome some of the gap between production and consumption of edible oil (Ghallab and Sharaan, 2002).

The low genetic variability and low yielding varieties play the most important factors for its low production. Therefore, availability of genetic variability is the prerequisite for any breeding program and is imperative for developmental or improved varieties of *Brassica* oilseed.

Induced mutation has been extensively used besides conventional methods for creating new genetic variation in crop plants. Literature revealed numerators mutant varieties of different crops with improved agronomic traits which have been developed and released to the farmers for general cultivation all over the world (Maluszynski *et al*., 2000). Mutagenesis technique has also been successfully employed in rapeseed and mustard by the plant breeders (Javed *et al*., 2000) to alter the genetic architecture of plant and isolate the possible mutants with desired plant characters such as plant height, number of pods per plant, number of grain per pod, 1000-grain weight, grain yield, oil content and disease resistance (Mahla *et al*., 1991; Rehman, 1996; Shah *et al*., 1998 & 1999; Javed *et al*., 2000).

Gamma rays generally induce cytological, biochemical, physiological, morphological and genetically changes in cells and tissues (Kiong *et al*., 2008). The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kovacs and Keresztes, 2002).

Different beneficial mutations have been developed in agricultural crops by using gamma irradiations. In barley, high yielding variety with early maturity, high protein contents and stiff straw was developed by the application of mutation breeding techniques (Gustafsson *et al*., 1971). Meanwhile, Shah *et al*. (2001) reported a new oil seed *Brassica napus* L cv. ABASIN-95 induced by gamma rays and the resulting new variety was high yielding, resistant to *Alternaria blight* and

white rust. Khatri *et al*. (2005) developed three high grain yielding and early maturing mutants by exposing seeds of *B. juncea* L. cv. S-9 to gamma rays and EMS.

Different molecular marker techniques have been used to study the genetic diversity among the genus *Brassica*. Markers assisted breeding is now used to enhance flexibility, choice of donor or breeding material of classic breeding program in genetic improvement of plant (Marjanovic *et al*., 2009; Ozbek and Gidik, 2013).

The present study was undertaken to induce some desired variability for agronomic and molecular characteristics and to estimate correlation and genetic diversity attributes in three *B. napus* genotypes (Serw 4, Serw 6 and Pactol) through mutagenesis induction using gamma rays.

MATERIALS AND METHODS

Plant material

In this study, three genotypes (Serw 4, Serw 6 and Pactol) of *Brassica* obtained from Agronomy Department, Faculty of Agriculture, Fayoum Univ., were used in this experiment.

Gamma irradiation

Seeds of the three genotypes (Serw 4, Serw 6 and Pactol) were exposed to gamma rays at different doses; control, 150, 300, 450 and 600 Gy.

Irradiation was performed using a Gamma cell 200 apparatus equipped with a ^{6o}Co γ source with average dose rate of 0.7 Gy/min. at National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt.

Seed germination

Germination percentages were recorded in the laboratory, using Petri dishes containing wet filter papers after three days of germination. Thirty seeds were sown in each dish and three dishes were used for each treatment.

M_2 generation

Radiated and untreated seeds were planted in the field, at Faculty of Agriculture farm, Fayoum University, Fayoum, Egypt, during the successive growth seasons from 2011 to 2013.

Self-pollinated seeds from M_1 generation for each treatment were planted in the field to obtained M_2 seeds. Seeds germination, plant height, No. of branches, No. of siliquas, seed yield per plant (g), and the morphological changes were observed in the M_2 generation). Oil and protein concentration were determined by nuclear magnetic resonance (Granlund and Zimmerman, 1975).

DNA isolation and PCR amplification

The genomic DNA of plant was extracted from leaves. Preparation of genomic DNA and separation of fragments by agarose gel electrophoresis was per-

formed as described by Sambrook *et al*. (1989). DNA concentration was quantified by visual comparison of DNA standard on ethidium bromide-stained agarose gel. Polymerase Chain Reaction (PCR) technique was performed with *Taq* polymerase (Fermentas). A set of RAPD primers was used (Table 1). PCR was performed in 50 µl reaction mixture containing 50 ng of template DNA, 2 µl (15 pmoles) of primer, 200 µM of each dNTP, standard buffer (supplied with the *Taq*) containing 1.5 mM $MgCl_2$, and 1 U of Taq DNA polymerase, in thermal cycler, as follows: initial denaturing step 94°C for 3 min., then (45 cycles) comprised a denaturing step at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min followed by final extension 72°C for 10 min. PCR products were separated in 1.5% agarose gel, stained with ethedium bromide and visualized under UV light.

Statistical analysis

PCR amplification products (bands) were scored as 1 (present) or 0 (absent) in each primer and the percentage of polymorphic bands was calculated. Reproducible and clearly distinguished bands were used in the analysis. Genetic similarity among all genotypes based on RAPD markers was calculated using the similarity index, $s = 2N_{ab}/(2N_{ab} + N_a + N_b)$, where N_{ab} is the number of bands shared by genotypes (a) and (b) in each pairwise comparison, and N_a and N_b are the number of bands present in the respective genotypes. Phylogenetic tree was constructed depending on the results of PCR products. The analysis of correlation and variance was carried out using the SPSS program.

RESULTS AND DISCUSSION

Nature and amount of genetic variability generated in different traits after exposure to gamma rays is presented in (Tables 2-4).

Seed germination

High doses of gamma rays were decreased the percentage of germination in all genotypes in compare with the control, but the low doses had no significant effect (Table 2). Similarly, Ayehband and Afsharinafar (2012) reported that the germination percentage was significantly decreased with increased gamma doses in *Amaranth* and Emerani *et al*. (2013) in corn. These results may be due to the effect of gamma radiation on the DNA and meristematic tissues of the plant, which is in agreement with those obtained by Boncheol and Maluszynski (1997) on barley and Cheema and Atta (2003) on rice.

Plant height

M_2 plants of genotypes Serw 4 showed higher stability in comparison with genotypes Serw 6 and Pactol. In genotype Serw 6, gamma irradiation significantly increased the plant height at 300 Gy compared to the control, but it was significantly decreased with 600 Gy. Genotype Pactol has general tendency to increase plant height in all treatments (Table 3). Reduction in plant height due to gamma

rays irradiation has been reported in different crops; Sarawgi and Soni (1993) on *Oryza sativa*; Sareen and Koul (1994) on *Plantago ovata* and Badr *et al*. (2004) on *Gomphrena globosa*.

The analysis of variance for the effects of different doses of gamma irradiation revealed fluctuations of induced variability in genotypes under study across different treatments. Variability in plant height with gamma irradiation had been reported by Charumathi *et al*. (1992) in black gram. Plant height is widely used as an index in determining the effect of chemical and physical mutagens on biological organism (Konzak *et al*., 1972). With regard to genotype-treatment interaction, a differential behavior was noted among varieties for this character.

The effect of irradiation on plant height might be due to their effect on DNA causing mutation and subsequently on the enzymes' synthesis which affect the metabolism activity and may be due to the different genetic material and environmental conditions.

Number of branches

Branches number of treated plants was increased, but no-significant compared with the control in genotypes Serw 4 and Pactol. On the other hand, the branches number of Serw 6 genotype was decreased with higher doses (Table 3). This results in agreement with the finding of Yaqoob and Ahmed (2003) in *mung beans* and Majeed *et al*. (2010) in *Lepidium sativum*.

As regards to the branching behavior of treated plants in M_2 generation, Table (3) indicates that there was no consistency in the number of branches in response to the increase in radiation dose. The effect was predominant in higher doses against the lower ones. In some cases, the number of branches increased in genotype Serw 4 and Pactol as compared to genotype Serw 6. Maximum branching was observed at higher doses, 600 Gy in genotypes Serw 4 and Pactol. At genotype Serw 6, the branching was markedly inhibited and a minimum number of branches were recorded at 600 Gy. Present findings are supported by similar reports in certain other plants; Malaviya (1984) and Mathur (1990) were observed that the number of branches was significantly decreased with increased dose of gamma rays in lentil and buckwheat. Where Shah *et al*. (1990) reported that the number of primary branches in rapeseed was increased when using gamma rays.

The effect of gamma radiation on inducing branching may be due to its effect on the production triggering growth hormones (Chandorkar and Dengler, 1987).

Number of siliquas

The high dose levels (450 and 600 Gy) reduced the number of siliquas per plant as compared with control in all genotypes but not significant in Serw 4 and Pactol, whereas the low doses (150 and 300 Gy) increased the number of siliquas per plant of all genotypes, especially in genotype Serw 6 (Table 3). An increase

in siliquas per plant after gamma rays-treatments has also been reported by other researchers in *Oleifurous brassica* (Shah *et al*., 1990; Khatri *et al*., 2005).

Seed/plant

Analysis of variance showed no significant difference for seed/plant between the control and the doses of gamma irradiation in Serw 4 genotype, while as decreased significantly with high doses in Serw 6 genotype. Pactol had different compartment, the high does increased the seed yield per plant (Table 3). Among the five doses and three genotypes, 450 Gy and Pactol produced the highest seed/plant (Table 3). Rahimi and Bahrani (2011) reported grain yield increased in response to application of gamma irradiation by 5% more than control but the high doses of gamma irradiation were reported to be had harmful effect.

Protein and oil content

Gamma rays had no significant effect on protein content of seeds in genotypes Serw 4 and Pactol but in the genotype Serw 6, protein content decreased about 35% at dose level 600 Gy compared with the control (Table 4). Our results are supported by previous published studies that reported decrease in protein production by gamma irradiation which may induce structural alteration of protein and denaturation and change their functional properties (Gaber, 2005; Chamani *et al*., 2009; Taghinejad *et al*., 2009).

Oil content is a primary and an important component of oilseed crops. All the genotypes under evaluation had tendency to increase the oil content with gamma ray doses but not significant. Barve *et al*. (2009) developed a high oil content mutant (NUDH-YJ-6) with 4.3% more than the control while Rahimi and Bahrabi (2011) reported that 100 Gy gamma rays produced the highest oil seed content, and the increase in gamma rays doses more than 100 Gy decreased oil seed.

The interaction between cultivar and gamma ray dose levels had no significant effect on oil percentage (Table 4). Among the five gamma rays dose levels and the three *Brassica* genotypes, 300 Gy and Pactol produced the highest oil content (46.7%). The results demonstrated a negative correlation between oil and protein content.

RAPD-PCR analysis

RAPD-PCR was used for the detection of DNA profile changes due to treatments with gamma rays (control, 150, 300, 450 and 600 Gy). Six primers out of twelve random 10-mer primers successfully amplified DNA fragments from *B. napus* DNA samples (Table 1). The results indicated the occurrence of structural changes in the treatment with six primers in the three genotypes. A total of 124 fragments were visualized across the six primers, 59 polymorphic bands were observed (Fig. 1). The percentage of polymorphism in genotypes (Serw 4, Serw 6 and Pactol) was (34.1%, 63.6% and 43%, respectively). The high polymorphism in genotypes Serw 4, Serw 6 and Pactol was

22, 36.4 and 30.7%, respectively, with high dose level of gamma rays (600 Gy). The result of RAPD analysis indicated the presence and disappearance of bands in response to treatments with doses of all gamma irradiation and correlated with the doses. Wendt *et al*. (2001) who used the RAPD markers to study the effect of gamma radiation on potato observed changes in the DNA bands and Ganapathi *et al*. (2008) on banana. The changes in the RAPD pattern of the present investigation were the appearance or absence of bands with variation in doses and genotypes. These effects might be due to the structural rearrangements in DNA caused by different types of DNA damages.

Danylchenko and Sorochinsky (2005) reported that, the appearance of new bands is usually resulting from different DNA structural changes such as breaks, transpositions, deletion …, etc.). Therefore, the RAPD genetic variability will provide useful information for breeding and has been successfully used to distinguish between cultivated plants (Chandra *et al*., 2010).

The tree was conducted depend on the results of PCR products showed the high similarity between the control and the lowest dose but the highest dose appear alone (Fig. 2). Additionally, Fig. (3) demonstrated the similarity between the interactions of six RAPD-PCR markers and five gamma rays dose levels of the three *Brassica* genotypes. Among different markers used and the five gamma rays doses and three *Brassica* genotypes, 600 Gy and genotype Pactol produced high diversity in separate cluster and the rest in other cluster. The results of the tree revealed highly similarity with the traits were measured.

Correlation studies

The correlation coefficients generally highlight the pattern of association among seeds/plant components and growth attributes, depicting how yield, as a complex character is expressed. Seeds/plant is positively correlated with the No. branches and No. siliquas (0.671** and 0.505**, respectively), indicating that seeds/plant is highly affected by the number of branches and siliquas. No. siliquas showed positive correlation with plant height and No. branches (0.455** and 0.529**, respectively) which revealed that higher genotypes would be high yielding. On the other hand, protein content illustrated negative correlation No. of branches and oil content (-0.224* and -0.766**, respectively), and high positive one with plant height (0.649**) (Table 5). Similar findings have also been reported (Das *et al*., 1984; Pathak *et al*., 1986; Khatri *et al*., 2005).

SUMMARY

This study was conducted to evaluate genetic diversity of the effect of gamma irradiation on three *Brassica* (*Brassica napus*) genotypes; Serw 4, Serw 6 and Pactol using morphological, physiological and molecular traits. In general, the best dose was the application of 300 Gy which stimulate plant growth to increase its ac-

tive substances productivity. Among the five doses and three genotypes, 450 Gy and Pactol produced the highest seed/plant. For breeding purpose, moderate gamma rays with low physiological effect and strong genetic effects are desirable. The application of RAPD-PCR technique showed high similarity between control and the lowest dose (150 Gy) in genotypes Serw 4 and Pactol and between 450 and 600 Gy in genotype Serw 6. Depend on the RAPD-PCR and agronomic data; Serw 4 and Pactol are more related than Serw 6. The effect of gamma rays was more effective in Serw 6 rather than genotypes Serw 4 and Pactol. The higher genetic diversity index should be used as potential donor materials in breeding programs. There is, therefore, possibility for further improvement in *B. napus* mediated induced mutations, leading to a genetic improvement of a specific trait and the selection of economically important mutants.

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Table (1): List of operon primers and their sequences.

Primer	Sequence
OP A-01	CAGGCCCTTC
OP A-04	AATCGGGCTG
OP A-05	AGGGGTCTTG
OP A-06	GGTCCCTGAC
UBC-223	GGGCAGGGGA
UBC-234	GGGCGCGAGT

Table (2): The percentages of seed germination of (Serw 4, Serw 6 and Pactol) genotypes of *B. napus* with different doses levels of gamma rays.

Irradiation dose (Gy)	Germination %		
	Serw 4	Serw 6	Pactol
Control	99 a	100 a	100 a
150	98 a	96 a	98 a
300	96 a	95 ab	98 a
450	90 b	88 bc	90 b
600	88 c	86 c	86 c

a, b, c,…..Means within same column followed by different letters are significantly different at P<0.05. Values are means of three replicates

Table (3): Plant height, no. of branches/plant, no. of siliquas/plant and seed/plant (g) among genotypes and treatments.

Irradiation Doses (Gy)	Plant height (cm)			No. of branches			No. of siliquas			Seed/plant (g)		
	Serw 4	Serw 6	Pactol	Serw 4	Serw 6	Pactol	Serw 4	Serw 6	Pactol	Serw 4	Serw 6	Pactol
Control	139 a	141 b	103 a	6.0 a	6.0 a	5.0 a	460 a	765 b	765 a	24.1 a	22.5 ab	30.9 ab
150	129 a	145 b	128 a	6.2 a	6.2 a	5.8 a	461 a	820 b	674 a	22.5 a	24.8 ab	23.9 b
300	133 a	176 a	141 a	6.2 a	6.6 a	6.6 a	475 a	980 a	767 a	26.6 a	29.2 a	30.3 ab
450	122 a	146 b	117 a	6.0 a	5.2 a	8.4 a	451 a	477 c	712 a	26.6 a	15.5 b	42.6 a
600	144 a	100 c	130 a	7.4 a	4.6 a	8.0 a	448 a	388 c	708 a	22.5 a	14.5 b	37.7 ab

a, b, c,…..Means within same column followed by different letters are significantly different at P<0.05. Values are means of three replicates

Table (4): Protein and oil content among genotypes and treatments.

Irradiation doses (Gy)	Protein content %			Oil content %		
	Serw 4	Serw 6	Pactol	Serw 4	Serw 6	Pactol
Control	24.6 a	29.9 a	24.2 a	43.7 a	39.1 a	45.5 a
150	24.4 a	29.7 a	24.6 a	43.8 a	39.1 a	44.7 a
300	22.8 a	29.7 a	24.1 a	45.4 a	39.1 a	46.7 a
450	22.7 a	27.8 a	25.0 a	45.2 a	41.1 a	46.1 a
600	24.5 a	19.5 b	23.9 a	44.0 a	42.6 a	46.3 a

a,b,c,…..Means within same column followed by different letters are significantly different at $P<0.05$; Values are means of three replicates

Table (5): Correlation among the evaluated traits of brassica genotypes with different treatments.

	No. of branches	No. of siliquas	Seed/plant	Protein %	Oil %
Plant height	0.130	0.455**	0.105	0.649**	-0.467
No. of branches		0.529**	0.671**	-0.224*	0.285
No. of siliquas			0.505**	0.318	-0.173
Seed/plant				0.057	0.047
Protein %					-0.766**

** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level.

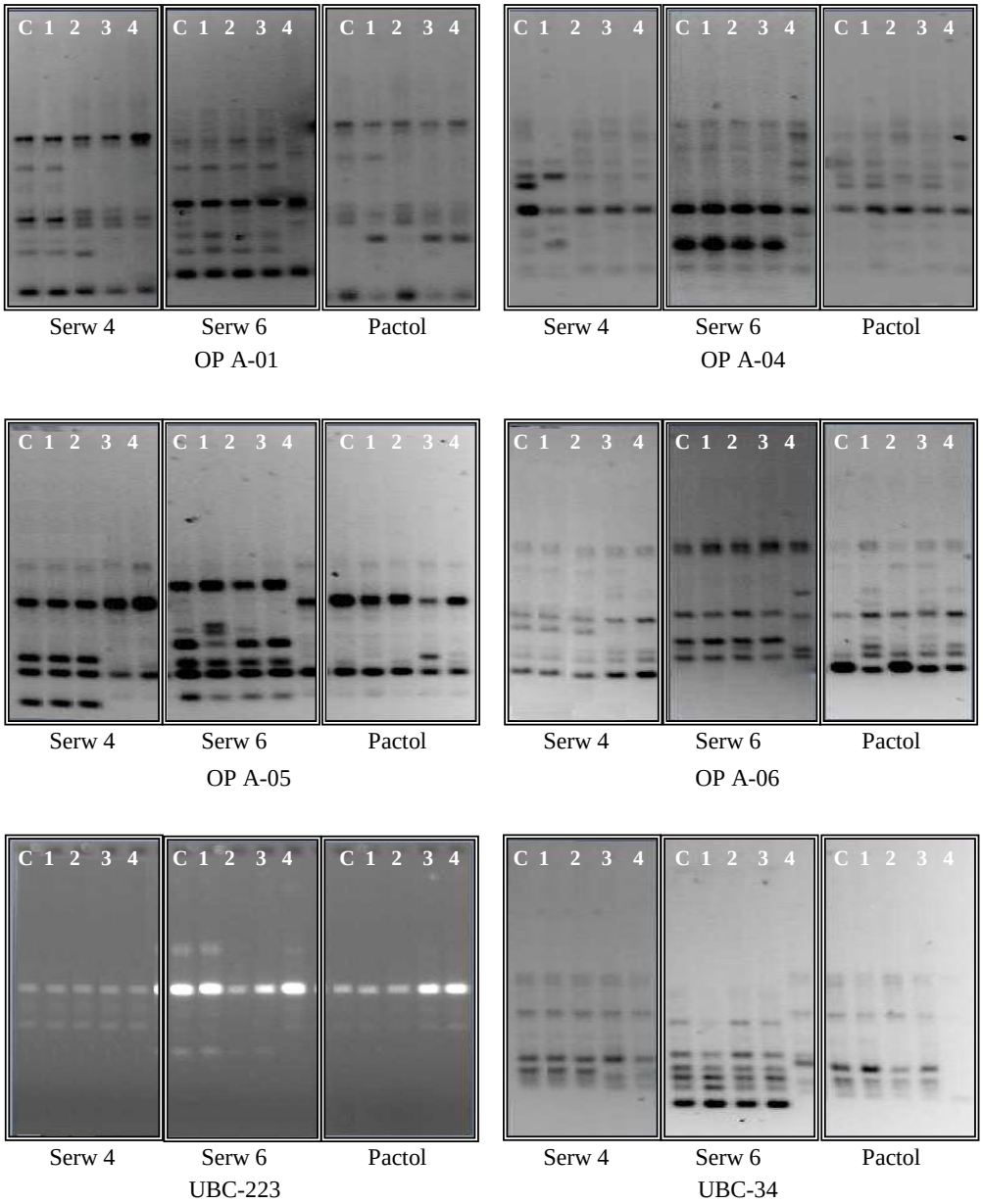

Fig. (1): RAPD fingerprints of the three *B. napus* genotypes (Serw 4, Serw 6 and Pactol) with five doses of gamma irradiation generated by six primers (OP A-01, OP A-4, OP A-05, OP A-06, UBC-223 and UBC-234). C= control, 1= 150 GY, 2= 300 Gy, 3 =450 Gy and 4= 600 Gy.

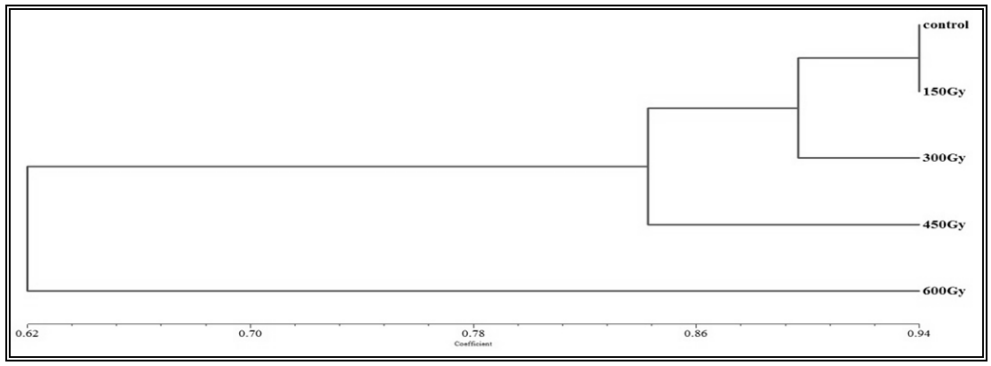

Fig. (2): Dendrogram among the five gamma rays doses of the (Serw 4, Serw 6 and Pactol) *Brassica* genotypes produced by RAPD-PCR bands.

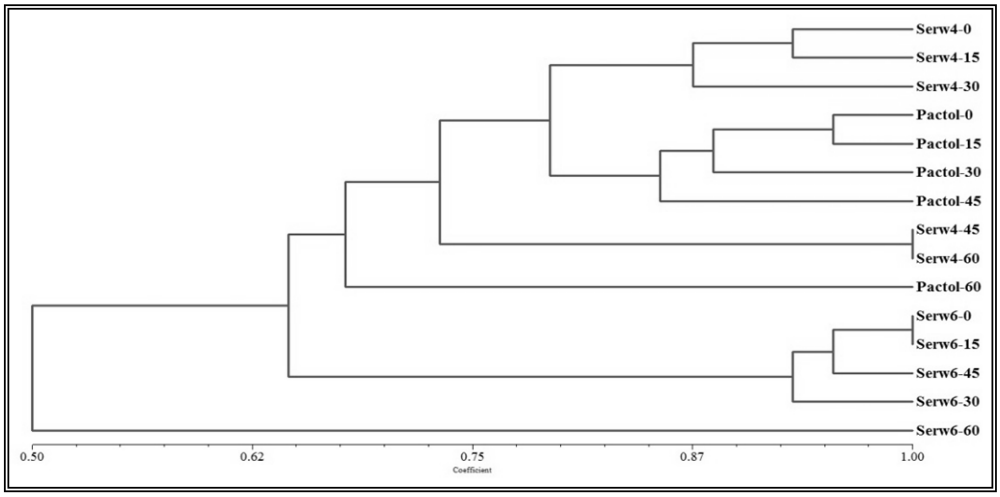

Fig. (3): Dendrogram among the five gamma rays doses and (Serw 4, Serw 6 and Pactol) *Brassica* genotypes produced by RAPD-PCR bands.