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Investigating the Genetic Diversity Arising from Two Physical Mutagens (Gamma Ray, Electron Beam) Using RAPD Markers in the Bread wheat (*Triticum aestivum L.*) Plant, Roshan Cultivar

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ABSTRACT

This article aims at investigating the genetic diversity arising from two physical mutagens (gamma ray, electron beam) with different doses (50-400 Gy) and two different levels of humidity (6.5% and 12.5%) in the (*Triticum aestivum* L.) plant in cultivar Roshan, using Random Amoplified Polymorphism DNA (RAPD) markers. Maximum and minimum average rates of heterozygosity were obtained in the second and the fifth populations (0.220 and 0.022), respectively. Based on the obtained results, the highest genetic distance or the lowest genetic similarity is observed between M1RE population (first generation mutant lines mutated by electron) and M2RG population (second generation mutant lines of the Roshan species mutated by gamma ray). The lowest genetic distance or the highest genetic similarity was found between M2RG (second generation mutant lines of the Roshan species mutated by gamma ray) and M2RE (second generation mutant lines of the Roshan species mutated by electron) populations. Five mutant lines and Roshan species witnesses were investigated based on common genetic values, using UPGMA cluster analysis. Cutting the obtained dendrogram from the similar point (0.84), three groups were achieved. Based on the obtained results, inter-population diversity is more significant than intra-population diversity.

KEYWORDS: RAPD, Gamma Ray, Electron Beam, Triticum aestivum

1. INTRODUCTION

Bread wheat (*Triticum aestivum L. em. Thell*) is one of the most ancient and prominent cereals and plays a strategic role for most of the world population. It is the main food of two billion people (36% of the whole world population) [1]. Bread wheat (42 = 6x = 2n) is a species of autogamous hexaploid (AABBDD) and a mixture of genomes of three old diploid species [2, 3, 4]. Different species of wheat are cultivated in different soils and climates around the world. Considering the fact that various wheat genotypes are threatened by biological and non-biological pressures, more wheat might be produced in different climates and more profitability might be achieved through optimal cultivation based on genetics [5].

Direct application of mutation especially to correct one or two recognizable characteristics of agricultural variety, is a useful complement in correcting vegetables. The main advantage of mutation is that the original variety is scarcely changed but favorable characteristics are achieved [6]. Vegetative cells reaction to physical and chemical mutagens is one of the biological, environmental and chemical factors which can change mutation effectiveness (mutation to dose ratio) and applicability (ratio of effective mutations to chromosome damages or abnormalities) [7, 8]. Physical mutagens include ionizing rays like X, gamma, beta and … [6]. Ionizing rays enter cells and tissues and react with different atoms and molecules and produce free radicals in cells. These radicals can damage or change important intracellular parts [9]. Mashev et al. [10] observed a significant decrease in wheat seed performance at doses higher than 0.10 KGy of gamma radiation. On the other hand, doses lower than 0.025 and 0.01 KGy of gamma ray increased seed performance. In his extensive study, Who [11] revealed that nutritive macromolecules (Carbohydrates, proteins) are relatively resistant to gamma radiation dose 10 KGy. Lee et al. [12] reported that gamma ray affects proteins through conformation changes, amino acids oxidation, covalent bonds breakdown and free protein radical formation. Zhu Hong and et al. [13] realized that electron rays of high energy pulse are not so much effective in direct deconstruction of cellular layer. On the other hand, electron beams of low doses improve the activity of celulase enzyme especially in aqueous environments. Yet, high doses of ray decreases enzyme activity. Alberti et al. [14] proved cellulose crystallization decrease in doses higher than 100KGy electron beam. They realized that crystallization index of cellulose microcrystalline in cotton and wool decreases 12 % with doses 200 KGy.

Genetic diversity estimation through morphological features is deficient in that the estimated features are limited and highly influenced by environmental factors. Molecular markers are more useful instruments in recognizing genetic diversity. This method is not influenced by environmental factors and does not need background genealogical information.

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Molecular markers are classified into two main groups:

1) Protein markers: these markers detect polymorphism at protein level but are strongly influenced by environmental factors [15,16,17,18].

2) DNA markers: these markers detect polymorphism at DNA level.

The main advantage of DNA markers is that they provide information regarding genome sequences of isoenzymes or non-enzyme proteins such as supply enzymes and their sequences [4, 15, 19, 20, 21, 22]. Many recent studies have focused on developing new methods of analysis using molecular markers, several of which have been based on PCR and genomic DNA [23]. PCR is the first and perhaps the most important system applied for increasing target molecules [24, 25, 26]. PCR-based methods using intentional primers include RAPD, AP-PCR (Arbitrary Primed Polymerase Chain Reaction) and DAF (DNA Amplification Fingerprinting) [15]. Being first applied by William [27], RAPD technique was then introduced by Welsh and McClelland [28] in DNA finger printing [29, 30]. RAPD molecular markers have different applications such as germplasm description, genome organism investigation, genetic diversity investigation, philogenetic analysis, genetic maps preparation and investigating genetic positions involved in quantitative trait loci controlling (QTC). Bozari [31] for detecting the genotoxic effect of one Triflurali pesticide species on corn, Pal Pepo [32] to investigate genetic variation in the number of lines and hybrids of maize, Ngezahayo [33] to investigate somaclonal diversity of nucleotide sequence in rice plant (*Oryza sativa*), Asif [5] for genetic analysis of six corn hybrids (*Zea mays*) and nine different bread wheat genotypes, Bered [34] to investigate the genetic diversity of bread wheat germplasm, Pakniyat [35] for investigating genetic diversity and dry resistance markers in 20 bread wheat species (*Triricum aestivam* L.), Nematzadeh [36] for determining the genes involved in controlling taste and odor in rice plant through F2 and F3, Naghavi [37] for determining genetic relationships among 36 wild diploid wheat populations (*Triticum boeoticum*)[38], RAPD markers were used. As a result, high genetic diversity of a population increases natural and artificial selection ranges. Recognizing the genetic diversity of agricultural plants corrected varieties is necessary. Hybridation of species with higher diversities leads to producing new lines and prevents genetic erosion [30].

2. MATERIAL AND METHODS

Bread wheat (*Triricum aestivam L.*) seed of Roshan species were prepared from the agricultural, medical and industrial research center of Karaj nuclear energy. To investigate the effect of humidity level and doses of two physical mutagens(gamma ray and electron beam) in producing genetic changes, specific amounts of wheat seed were mixed with purified water(10% of seeds weight) and their humidity levels were set at two different ranges [6.5-7% and 12.5-14%]. Non-radiated Seeds with 3.5-4% humidity level were considered as witness or control samples. Wheat seeds humidity was set at an appropriate level. The first portion of seeds was immediately exposed to gamma ray of Co_{60} source with doses of 50 Gy, 100 Gy, 200 Gy, 300 Gy and 400 Gy; at the atomic energy research center. The second portion was transferred to the electron ray center of Yazd atomic energy organization and was exposed to different doses of electron beam (100 Gy, 200 Gy, 300 Gy and 400 Gy). Seeds were carried on dried ice to keep their humidity level. Having been radiated by two physical mutagens with different doses, seeds were cultivated in soil block under greenhouse conditions with approximate distances of 20cm. seeds were watered weekly based on their needs.

DNA extraction using DArT method:

DNA samples of fresh leaves of plantlets 15 days picked and transferred to freezer-70 were extracted using DArT protocol [39]. To evaluate the extracted DNA quality, was used from electrophoresis with agarose gel 0.8% in the electrical field of 120v. Furthermore; to investigate DNA quantity, Nanodrop machine was used at wavelengths of 230, 260 and 280 nanometers.

RAPD-PCR experiment:

RAPD-PCR reaction requirements:

A) primers:

A total number of 30 specific 10- nucleotide primers were applied for RAPD reaction. All used primers were prepared from Operon Technology Company (Alameda, CA). Then, a primer solution of 10µM was prepared. Primers names and equivalents are mentioned in Table 2-1.

B) Mastermix preparation and PCR stage regulation :

The final volumes and contents of PCR components for a 25µL reaction , 17.5µl Steriled ddH_2o, 2.5µl PCR buffer (10X), 2µl $Mgcl_2$ (10mM), 0.5µl dNTP (10mM), 1µl primer (10µM), 0.5µl Taq DNA polymerase (5U/µl), 1µl DNA; was used. All PCR experiments were conducted in thermocycler machine (Mastercycler gradient (Corbet) model) (Table 2-2).

PCR products electrophoresis:

The products reproduced during PCR reaction were separately inserted on the gel agarose 1%- prepared by Buffer TBE (1X) under voltage of 100v. It is worth mentioning that the molecular weight marker called 100bp DNA ladder plus the product of Fermentas company- was applied (Images 2-1 and 2-2).

Table 2-1: primers names and equivalents

Row	Primer	Sequence (5'⟶3')	Row	Primer	sequence (5'⟶3')
16	OPC-19	GTTGCCAGCC	1	OPA-9	GGGTAACGCC
17	OPD-4	TCTGGTGAGG	2	OPA-10	GTGATCGCAG
18	OPD-11	AGCGCCATTG	3	OPA-12	TCGGCGATAG
19	OPF-1	ACGGATCCTG	4	OPA-13	CAGCACCCAC
20	OPF-14	TGCTGCAGGT	5	OPA-14	TCTGTGCTGG
21	OPG-4	AGCGTGTCTG	6	OPA-18	AGGTGACCGT
22	OPG-6	GTGCCTAACC	7	OPA-19	CAAACGTCGG
23	OPR-2	CACAGCTGCC	8	OPA-20	GTTGCGATCC
24	OPR-3	ACACAGAGGG	9	OPB-05	TGCGCCCTTC
25	OPUBC30	CCGGCCTTAG	10	OPB-06	TGCTCTGCCC
26	OPUBC75	GAGGTCCCAA	11	OPB-08	GTCCACACGG
27	OPUBC78	GAGCACTAGC	12	OPB-11	GTAGACCCGT
28	71AB10G11	AGCGCCATTG	13	OPC-02	GTGAGGCGTC
29	72AB10G12	AGGGCGTAAG	14	OPC-15	GACGGATCAG
30	73AB10T13	CTGGGGACTT	15	OPC-16	CACACTCCAG

Table 2-2: PCR thermal plan for RAPD experiment

Stage	Temperature	Time	Cycle number
First denaturation	94^{oc}	3 minutes	1
Denaturation **Annealing**	94^{oc} 42^{oc}	30 seconds 30 seconds	35
Extension	72^{oc}	30 seconds	1
Final extension	72^{oc}	7 seconds	1

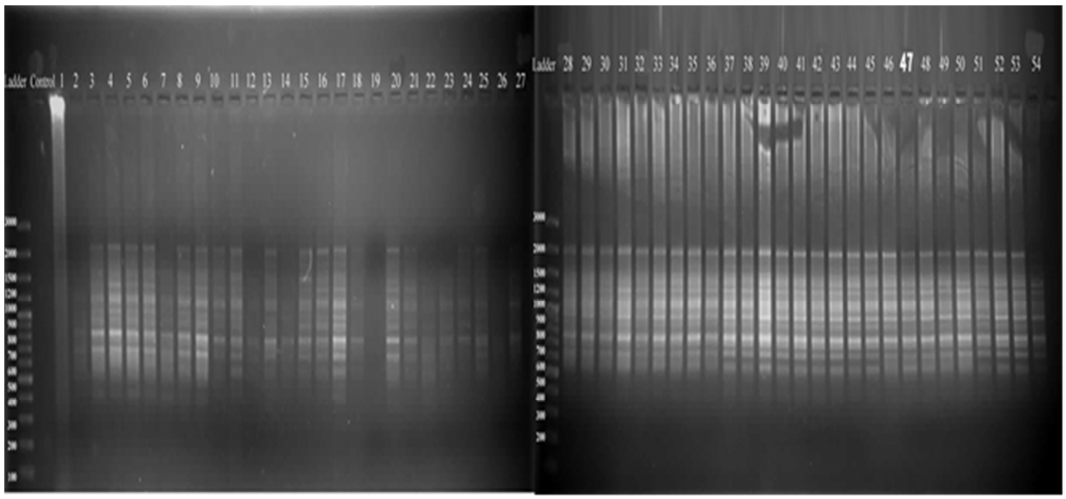

Image 2-1: the bands reproduced by OPA-20 primer

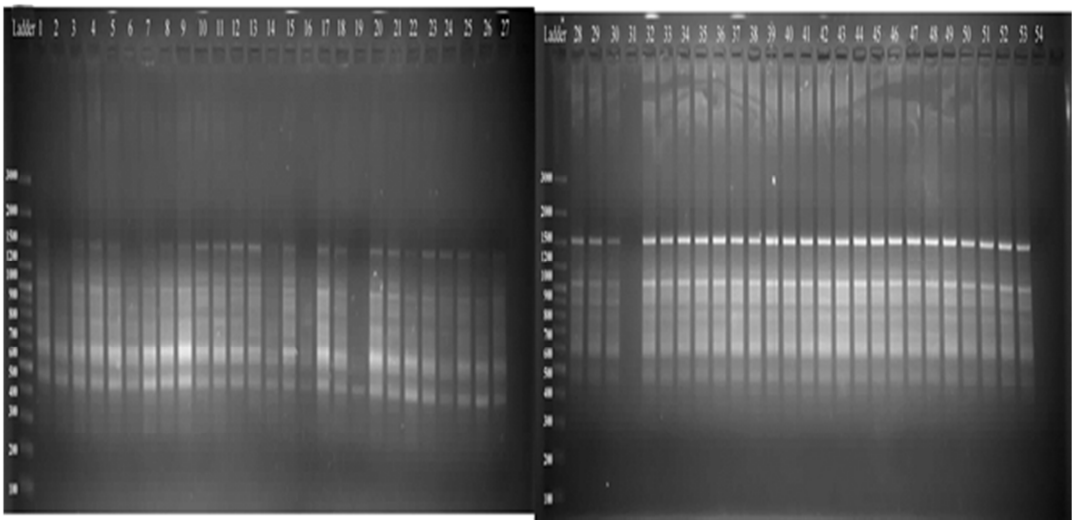

Image 2-2: the bands reproduced by OPA-13 primer

Statistical analysis

PARD data are in a binary mode. Code 1 indicates bands presence and code 0 indicates bands absence. To investigate molecular diversity and recognize heterotic groups in Tabasi bread wheat species, a total number of 26 Roshan samples including control samples, M1 and M2 mutant lines (Table 2-3) were investigated using 30 RAPD primers (Table 2-1) among which 22 primers revealed polymorphism status. Three softwares- NTYSYS-pc, POPGENE [40] and Genealex were used for statistical analysis and score allocation to bands. Different indexes were used for evaluating RAPD markers utility, diversity [41,42], distance; and genetic similarity of populations [43] which will be explained in turn. To evaluate genetic diversity using molecular markers information, several factors might be considered among which are: polymorphic information content (PIC), Heterozygosity (H) [44,45]. Polymorphic information content [46, 47] is an estimation of primers detection ability [43] considering alleles numbers and relative frequency [48, 49].

Table 2-3: mutant genotypes features, their numbers and abbreviation codes on electrophoresis gel

Number	Code	Genotype features
10	**RC1**	**Roshan witness, number 1**
11	M1RH6G50	First generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 50 Gy
12	M1RH6G100	First generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 100 Gy
13	M1RH6G200	First generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 200 Gy
14	M1RH6G300	First generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 300 Gy
15	M1RH6G400	First generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 400 Gy
16	M1RH12G50	First generation, Roshan species, 12.5% humidity, mutations of gamma ray dose of 50 Gy
17	M1RH12G100	First generation, Roshan species, 12.5% humidity, mutations of gamma ray dose of 100 Gy
18	M1RH12G200	First generation, Roshan species, 12.5% humidity, mutations of gamma ray dose of 200Gy
24	RC2	Roshan witness, number 2
25	M1RH6E100	First generation, Roshan species, 6.5% humidity, mutations of electron beam dose of 100 Gy
26	M1RH6E200	First generation, Roshan species, 6.5% humidity, mutations of electron beam dose of 200 Gy
27	M1RH12E100	First generation, Roshan species, 12.5% humidity, mutations electron beam dose of 100 Gy
37	RC3	Roshan witness, number 3
38	M2RH6G50	Second generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 50 Gy
39	M2RH6G100	Second generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 100 Gy
40	M2RH6G200	Second generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 200 Gy
41	M2RH6G300	Second generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 300 Gy
42	M2RH6G400	Second generation, Roshan species, 6.5% humidity, mutations of gamma ray dose of 400Gy
43	M2RH12G50	Second generation, Roshan species, 12.5% humidity, mutations of gamma ray dose of 50 Gy
44	M2RH12G100	Second generation, Roshan species, 12.5% humidity, mutations of gamma ray dose of 100 Gy
45	M2RH12G200	Second generation, Tabasi species, 12.5% humidity, mutations of gamma ray dose of 200 Gy
51	RC4	Roshan witness, number 4
52	M2RH6E100	Second generation, Roshan species, 6.5% humidity,mutations of electron beam dose of 100 Gy
53	M2RH6E200	Second generation, Roshan species, 6.5% humidity,mutations of electron beam dose of 200 Gy
54	M2RH12E100	Second generation, Roshan species,12.5% humidity,mutations of electron beam dose of100 Gy

3. RESULTS

Molecular variance analysis (AMOVA):

Molecular variance analysis (AMOVA) is a method for molecular diversity investigation in a species based on a hierarchical or nest model [50, 51]. Molecular variance analysis of data and molecular variance percent calculation in populations or among them is fulfilled by Genealex software (6.41 version) [50, 51].

Cluster analysis:

Cluster analysis is a general concept referring to a set of mathematical methods used for recognizing similarities among individuals. Members of a cluster are more similar to each other than members of different clusters. In one of the clustering methods, individuals are grouped based on their similarities. Individuals are grouped according to dendrogram and hierarchical diagrams. UPGMA is the most fashionable clustering method which is most applicable in genetic diversity analysis. All cluster analysis stages and dendrogram depiction is performed in NTSYS-pc 2.02e.

Cluster analysis confirmation (cophenetic correlation coefficient calculation):

The hierarchical diagram arising from cluster analysis implies data matrices but might never precisely express it. There are several methods for approving cluster analysis methods and evaluating similarity or distance criterions one of which is cophenetic correlation coefficient. This coefficient reveals what extent of initial data or input matrices (matrix of initial distance and similarity) has been reflected in dendrogram. Based on the depicted dendrogram, a new matrix is calculated which is called cophenetic coefficient. If the coefficient is higher than 0.8,

cluster analysis method has successfully applied initial distance or similarity in grouping individuals and populations. Cophenetic coefficient was calculated by NTSYS-pc software.

Principle coordinate analysis (PCOA):

The huge number of calculated variables is one of the fundamental problems in statistical analysis. Increases of variables number (electrophoresis bands number in case of molecular data) increased correlation coefficient numbers. The huge number of variables leads to numerous relationships. So, we need techniques to decrease data and abbreviate correlation matrix. Principle coordinate Analysis (PCOA) is one of the valuable notions of linear algebra. It is a simple method used for extracting necessary data from a wide range of complicated data. In this method, mixed data groups are decreased to more limited aspects and the structures responsible for data changes are switched [52].

4. DISCUSSION AND CONCLUSION

Genotypes (Roshan species and their mutant lines) were grouped using NTSYS ver-2.1 software, UPGMA method and complete linkage method. Similarity coefficients of Dice, Jacard and Simple Matching were also applied in this regard. To evaluate methods performances, cophentic coefficient was calculated for depicted dendrogram. Cluster analysis method was finally adopted using simple adaptation similarity coefficient and complete linkages method with maximum cophentic coefficient (r=0.885). Cutting cluster analysis diagram from 0.74 similarity point, the studied genotypes were classified into six main groups (Figure 4-1). Having grouped mutant lines and Roshan witness species using molecular data of RAPD markers, it might be concluded that the RAPD markers have effectively separated witness species from mutant lines. Furthermore, first and second generation mutant lines are classified into two separate groups. Mutant lines mutated by different methods (gamma ray and electron beam) have been separated into several subgroups. Witness species and mutant lines have been separated due to their different features. Mutation by gamma ray and electron beam has caused genetic changes at DNA level in the species. Due to their higher diversity, first generation mutant lines are classified into several groups (groups 3,4 ,5 and 6). They are placed in the same group at lower similarity points (0.60).

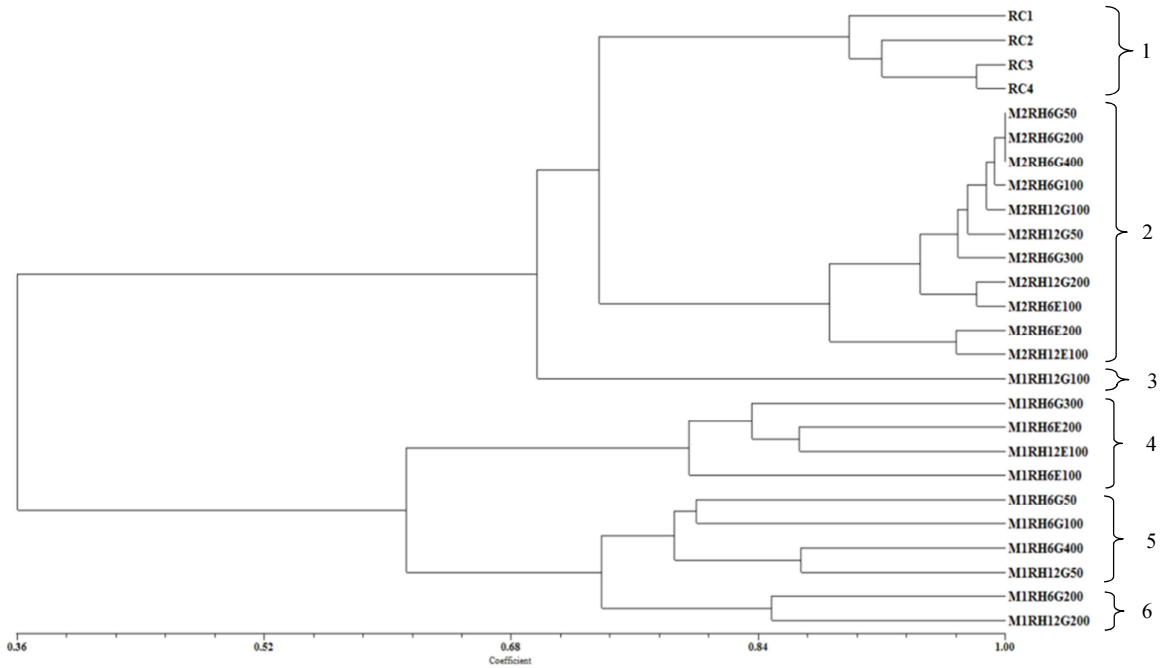

Figure 4-1: Depicted dendrogram for 22 mutant lines and four Roshan species witnesses, using simple adaptation similarity coefficient and complete linkage method

Iqbal [53] in determining genetic divergence degree of 7 bread wheat species, genetic similarity matrix of Nei and Lei achieved in 86.2-93% range. Raghunathachari [54] to determine the genetic diversity of in Indian aromatic rice germplasm, achieved a wide range of similarity from 25% to 77.5%. Kanawapee [55] in investigate genetic diversity of 30 rice plant species classified into five groups in terms of saltiness resistance, genetic similarity averages of 0.82 achieved. Bakatoushi [56] in investigate the genetic diversity of the growing winter bread wheat (near to high voltage transfer line), achieved a high genetic distance among high voltage-exposed individuals (0.33 and 0.89 domains) compared to those working in fields lacking high voltage lines with lower genetic distances (0.10 to 0.29).

Grouping Roshan witnesses and their mutant lines using principle coordinate analysis (PCOA):

Principle coordinate analysis was conducted using NTSYS ver-2.1 software. In principle coordinate analysis, the determined components (the first two or three components) account for a high percent of molecular data changes. The values related to principle coordinate analysis include: Eigen value, variance percent and cumulative variance. These values were calculated for the first fifteen components which account for 99% of changes, in Table 4-1. The determined components are independent. This is to say that the applied markers are not related to each other and are homogenously distributed on the studied wheat genotypes genomes.

Based on the two-dimensional diffraction obtained from the two first main components of principle components analysis, studied genotypes might be separated into different groups (Table 4-2 and Figure 4-2). These groups are a little different from cluster analysis grouping.

Table 4-1: Eigen values, variance percent and cumulative variance of the components obtained from principle coordinate analysis in Roshan mutant population and their witnesses

Component	Eigen value	Variance percent	Cumulative variance
1	19.14	73.64	73.64
2	3.41	13.10	86.74
3	0.68	2.61	89.35
4	0.54	2.09	91.43
5	0.35	1.35	92.79
6	0.32	1.24	94.03
7	0.26	0.99	95.02
8	0.22	0.84	95.86
9	0.18	0.68	96.54
10	0.16	0.60	97.14
11	0.15	0.56	97.70
12	0.12	0.46	98.16
13	0.10	0.38	98.54
14	0.10	0.38	98.92
15	0.08	0.30	99.23

Table 4-2: determining line names and numbers for their easier recognition in principle coordinate analysis

Number	Line name
1	Roshan witness 1
2	Roshan witness 2
3	Roshan witness 3
4	Roshan witness 4
5	First generation mutant with 6% humidity and gamma ray of dose 50 Gy
6	First generation mutant with 6% humidity and gamma ray of dose 100 Gy
7	First generation mutant with 6% humidity and gamma ray of dose 200 Gy
8	First generation mutant with 6% humidity and gamma ray of dose 300 Gy
9	First generation mutant with 6% humidity and gamma ray of dose 400 Gy
10	First generation mutant with 12% humidity and gamma ray of dose 50 Gy
11	First generation mutant with 12% humidity and gamma ray of dose 100 Gy
12	First generation mutant with 12% humidity and gamma ray of dose 200 Gy
13	First generation mutant with 6% humidity and electron beam of dose 100 Gy
14	First generation mutant with 6% humidity and electron beam of dose 200 Gy
15	First generation mutant with 12% humidity and electron beam of dose 100 Gy
16	second generation mutant with 6% humidity and gamma ray of dose 50 Gy
17	second generation mutant with 6% humidity and gamma ray of dose 100 Gy
18	second generation mutant with 6% humidity and gamma ray of dose 200 Gy
19	second generation mutant with 6% humidity and gamma ray of dose 300 Gy
20	second generation mutant with 6% humidity and gamma ray of dose 400 Gy
21	second generation mutant with 12% humidity and gamma ray of dose 50 Gy
22	second generation mutant with 12% humidity and gamma ray of dose 100 Gy
23	second generation mutant with 12% humidity and gamma ray of dose 200 Gy
24	second generation mutant with 6% humidity and electron beam of dose 100 Gy
25	second generation mutant with 6% humidity and electron beam of dose 200 Gy
26	second generation mutant with 12% humidity and electron beam of dose 100 Gy

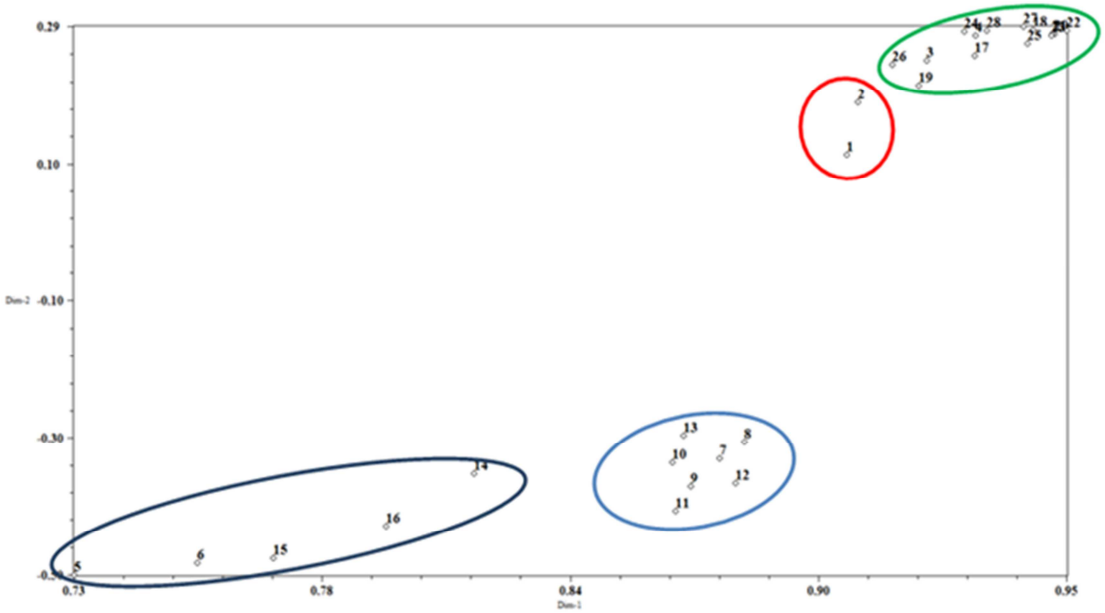

Figure 4-2: the two-dimensional diagram obtained from principle coordinate analysis of mutant lines and Roshan species witnesses

Obtained results of the reproduced samples in five populations of Roshan species mutant line and their witnesses:

Studied genotypes were separated into five populations and were subjected to population analysis by the molecular softwares of POPGENE ver 32 and Genealex ver 6.41.

In Table 4-3, data related to reproduced positions in five Roshan species mutant lines and their witnesses have been demonstrated. Band pattern of the five populations related to Roshan species mutant lines and their witnesses are demonstrated in diagram 4-1.

Table 4-3: data related to reproduced positions in five mutant populations and Roshan species.

Population	Line number	The average number of observed allels in each primer	The average number of effective allels in each primer	The average Shannon index in each primer	Average estimated Heterozygosity in each primer	Polymorphic positions percent
Population 1 (RC)	4	0.958	1.124	0.093	0.066	%14.81
Population2 (M1RG)	8	1.475	1.388	0.326	0.220	%59.88
Population3 (M1RE)	3	0.691	1.132	0.117	0.078	%20.99
Population 4 (M2RG)	8	0.969	1.039	0.031	0.022	%5.56
Population 5 (M2RE)	3	0.932	1.043	0.036	0.024	%6.17
Average	5.2	1.005	1.145	0.121	0.082	%21.48

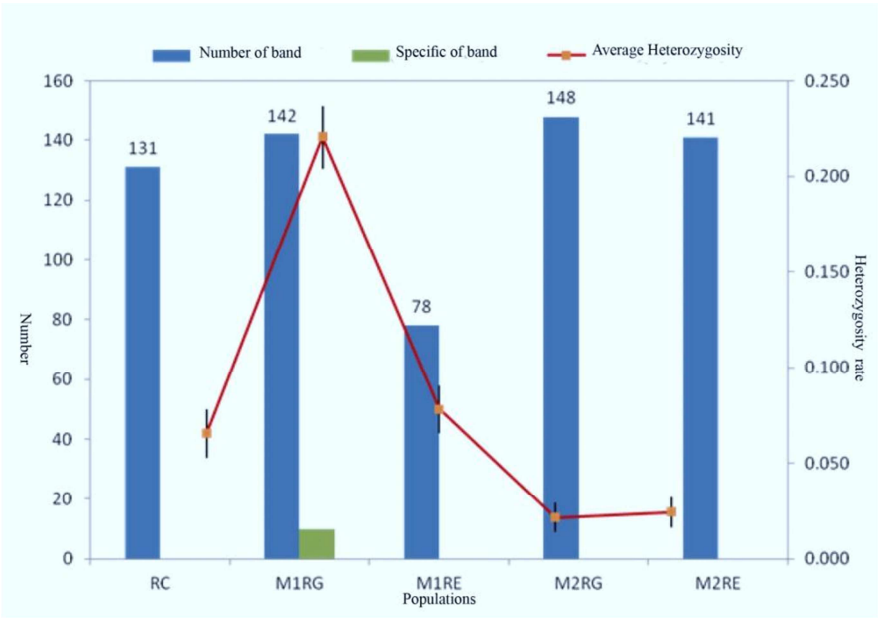

Diagram 4-1: band pattern of five mutant population and Roshan species witnesses

In genetic analysis of mutants resulted from gamma ray in rice, Morita et al. [57] indicated that the most frequent mutations induced by gamma ray in rice, leading to genetic variety, are omission mutations especially small omission mutations as little as one single pair or several ten bp or 10 Kbp.

Genetic similarity and distance among the populations related to mutant lines and Roshan species witnesses:

Genetic distance between two entities is the difference of two entities which might be explained by allelic diversity. In other words, this index describes the genetic differences among populations and varieties measurable by numerical quantities. Genetic similarity and distance among populations was measured by Nei method [42, 45] and the results were demonstrated in Table 4-4.

Bhutta [58] in determining divergence degree of seven bread wheat species (*Triticum aestivum*) bred in different Pakistan regions. Genetic similarity matrix was observed among genotypes at 84%-93% range. Ahmed [59] in investigate genetic diversity among 32 lines of modified hexaploid bread wheat that are culturing in the rainfed, genetic similarity coefficients was observed among genotypes at 0.81%-0.94% range.

Table 4-4: genetic distance and genetic similarity among the five populations related to Roshan species mutant lines based on Nei index

Populations	Population 1(RC)		Population 2(M1RG)		Population 3(M1RE)		Population 4(M2RG)	
	Distance	Similarity	Distance	Similarity	Distance	Similarity	Distance	Similarity
Population1 (RC)	0.000	1.000						
Population2 (M1RG)	0.276	0.759	0.000	1.000				
Population3 (M1RE)	0.484	0.616	0.149	0.862	0.000	1.000		
Population4 (M2RG)	0.167	0.846	0.505	0.604	0.831	0.436	0.000	1.000
Population5 (M2RE)	0.196	0,822	0.472	0.624	0.762	0.467	0.050	0.951

Cluster analysis of five populations related to mutant lines and Roshan species witnesses:

Cluster analysis of five populations related to mutant lines and Roshan species witnesses was conducted based on Nei genetic similarity amounts using UPGMA method. The obtained dendrogram is demonstrated in Figure 4-3. Cutting the diagram from 0.84 similarity point, three groups are obtained.

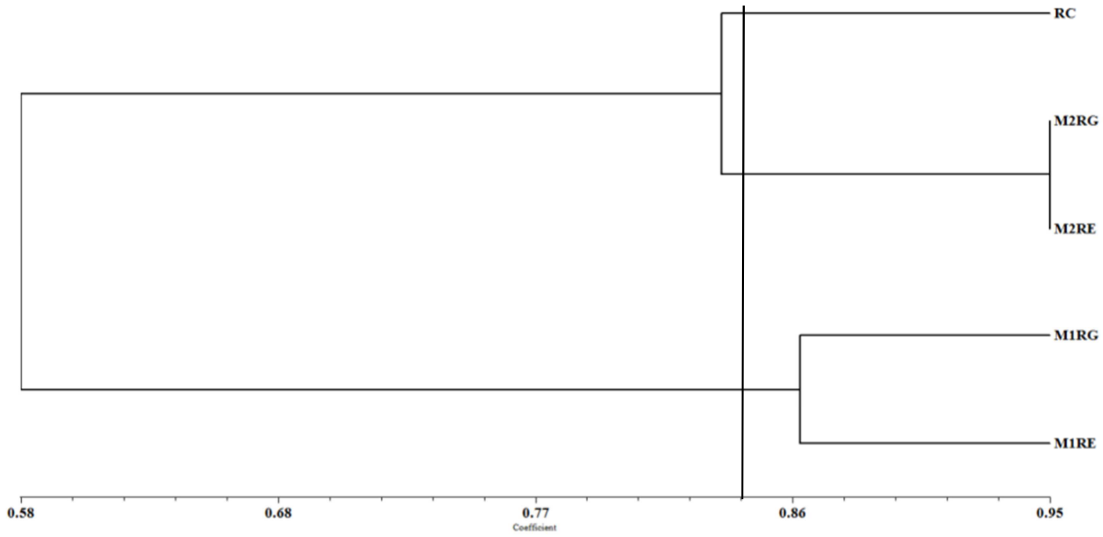

Figure 4-3: Dendrogram depicted using UPGMA method based on Nei genetic distance between mutant populations and Roshan type witnesses

Mitra [60] in molecular analysis of six hexaploid bread wheat types, In UPGMA dendrogram, six wheat types were grouped into two main clusters based in Nei coefficient. Haleam [61] in genetic analysis and RAPD polymorphic investigation of 11 different genotypes Durum wheat, Using dendrogram analysis, these 11 genotypes were placed into two clusters Rashed [62] applied RAPD primers to estimate genetic diversity of 30 hexaploid bread wheat varieties. UPGMA cluster analysis dendrogram classified varieties into two main clusters.

The obtained results of molecular variance analysis of mutant lines and Roshan witnesses (AMOVA):

Molecular variance analysis aims at investigating inter-population and intra-population diversity. Based on the obtained results, inter-population diversity is significant and higher than intra-population diversity. Intra-population variance accounts for 14% of the total diversity while inter-population variance accounts for 86% of

the total diversity. Obtained results of molecular variance analysis are demonstrated in Table 4-5. Chakarabarty [63] stated that RAPD analysis is now used not only for estimating genetic variety in mutants induced by gamma ray, but also for proper identification of new mutants/types for their legal protection, under the title of plant variety improvement.

Table 4-5 : molecular variance analysis of mutant lines and Roshan species witnesses

Change source	Degree of freedom	Sum of squares	Average of squares	Variance percent
Inter-population	4	377.61	94.40**	86%
Intra-population	21	309.62	14.74	14%
Total	25	687.23	--	

** Significant at 1% level of significance

Based on the results obtained in this study, 30 RAPD primers, 22 primers polymorphic bands with relatively good distribution among established genotypes, This indicates that appropriate selection markers for genetic variation in the genotypes this research. Cluster analysis using data from the investigated markers, RAPD, as well as a mutant strains in the Bread wheat (Roshan Cultivar) is separated.

Suggestions:

1) Use of appropriate mutant strains as modified crops.
2) Use the appropriate mutants in breeding program to cross.
3) Establish and Comparison of genetic diversity obtained with other doses of gamma radiation and electron beams used in this study.
4) Use more specific markers for measuring genetic diversity resulting from physical mutagens (Gamma ray and electron beam) as: SSR, RFLP, etc.
5) Detection and isolation of genes involved in plant resistance of wheat against radiation stress.

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