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MOLECULAR ANALYSES OF SELECTED TEA GENOTYPES IRRADIATED WITH GAMMA RAYS

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Abstract

Thirty six tea genotypes (M1-M36) along with three check genotypes (Qi men, P3 and Indonesian) treated with gamma irradiation were evaluated with help of Random Amplified Polymorphic DNA (RAPD) markers. All the genotypes were treated with 10 Kr gamma radiations and grown by using augmented design. A total of 8 RAPD markers were used for PCR amplification of all 39 genotypes. Maximum polymorphic bands were recorded. However 16 unique extra alleles were found in the experimental samples. The genetic similarity values varied among genotypes. The phylogenetic analysis classified all the genotypes into six diverged groups (I-VI). The groups (I-VI) contained 17, 8, 4, 2, 7 and 1 genotype, respectively. Maximum variability in the allelic pattern was observed in treated samples. The variability in band patterns might be due to the mutation. The 3D analysis identified 4 elite tea genotypes (Qi men, M19, M28 and M31). The important tea genotypes treated with gamma radiations were characterized at molecular level. However further characterization through SSRs or SNP markers are needed to check further genomic variability at different gamma radiation treatments.

Key words: Gamma radiation, Genetic variability, Molecular marker, Mutation, Tea genotypes.

Introduction

Tea is one of the important plant species belonging to the family Theaceae. The tea plant is full of multiple branches and has an evergreen color (Mong & Hsiech, 2007). Tea is used in our daily life and used as a chief source of drink. It provides body relaxation, removes body tiredness, and stimulates memory and metal stability (Mulky& Sharma, 1993). Green tea is also one of the important types of tea, prepared from by drying the leaves. The black tea is prepared by fermentation of dry leaves (Facciola, 1990).

The irradiation therapy is one the efficient and quick methods to produce changes in plants as compared to other physical treatments. As results of its treatment, the ionized energy enters rapidly to the polysaccharide granules (Ilyas & Naz, 2014). It brings rapid mutation per unit area. The gamma rays have a high penetration rate and are highly energetic (Kovacs & Keresztes, 2002). Different plant parts or whole plants are used for irradiation treatment. But mostly the seed part is predominantly used to bring efficient mutation in plants. In addition the treated seed sample has several advantages as it can easily be handled, stored, processed and maintained (Malik, 2009). In agriculture the radiation applications are used to control crops from pest attack, to bring genetic variability and to produce biochemical and physiological changes in plants (Howard, 1958). It can affect plant growth and development by bringing morpho-biochemical, physiological and molecular changes in cells or tissues (Ikram *et al.*, 2010). The morpho-biochemical markers are used in plants to identify and characterized the smart/elite genotypes (Nawaz *et al.*, 2015; Gul *et al.*, 2018; Gamar *et al.*, 2018; Jan *et al.*, 2018; Kumar *et al.*, 2018; Shinwari*et al.*, 2018; Akbar *et al.*, 2019). The morphological markers are affected by environmental factors, while the molecular markers show resistance to abiotic factors and provide efficient plant diversity (Shinwari *et al.*, 2013; Rehman *et al.*, 2015; Jan *et al.*, 2017; Ibrar *et al.*, 2018). Therefore, the present study was conducted to evaluate the selected tea genotypes at molecular level with recommended gamma rays treatment.

Materials and Methods

Plant materials and experimental design: The field experiment was performed at National Tea and High Value crops Research Institute (NTHRI) Shinkiari, Mansehra, Pakistan. The molecular analysis was performed at tissue culture lab, department of Botany, Hazara University, Mansehra. The 36 tea genotypes along with 3 check genotypes (Qi-men, P-3 and Indonesian) were used for molecular analysis.

The experiment was conducted by using augmented design by repeating the check genotypes. The plants were first grown in nursery then shifted to the field. The fresh seeds were mutated with at 10 Kr gamma rays. The DNA was extracted by following the method of Weining & Langridge (1992).

A total of 8 RAPD primers (Table 1) were used for PCR analysis to access molecular based variability among 39 tea genotypes. The PCR was conducted by preparing 25 µl master mix having ~50 ng genomic DNA, primer (0.25µM), dNTPs (200µM), 50mM KCl, 10mM Tris, 1.5mM $MgCl_2$ and Taq DNA Polymerase (2.5 units). The PCR condition was maintained as initial denaturation for 4 mins at 94°C (40 cycles), final denaturation for 1 min at 94°C, annealing for 1 min at 34°C and initial extension and final extension for 2 and 10 mins respectively, at 72°C. The PCR products were then confirmed on 1.5% agarose gel.

Table 1. Name, sequence and size (bp) of RAPD primers.

S. No.	Name	Sequences	Size
1.	AC – 05	GTTAGTGCGG	10
2.	AC – 07	ACGGAAGTGG	10
3.	AC – 08	TTGGGGGAGA	10
4.	AC – 09	AGAGCGTACC	10
5.	OPA – 05	AGGGGTCTTG	10
6.	OPA – 10	GTGATCGCAG	10
7.	OPA – 11	CAATCGCCGT	10
8.	OPB – 1	GTTTCGCTCC	10

Data analysis

The clear and sharp bands were used for Bivariate data analysis. The genetic distances were measured by using the method of Unweighted Pair Group of Arithmetic Means (UPGMA) Nei & Lee (1979). The genetic tree was constructed by using software of "Pop gene 3.2." The Principal Coordinate Analysis (PCoA) was performed to check the genotypes from a close angle by using software NTSYSpc 2.10 (Rohlf, 2000).

Results and Discussion

Genetic polymorphism among 39 tea genotypes: In current study 39 tea genotypes treated with 10 Kr gamma rays were screened by using RAPD markers. Maximum polymorphic bands were recorded with sizes ranging from ~100 to ~1200 bp (Fig. 1a-c). A total of 77 bands were recorded with 8 primers sets with an average 1.97 allele per genotype. A total of 16 extra alleles were recorded in treated plants as compared to control plants. The gamma irradiation can bring novel changes in the genomic sequence of any plant species. Maximum genomic variability was recorded among all tested genotypes at molecular level. In comparison with check genotypes, some diverged genotypes were recorded that can be the future lines for breeding programs. All the genotypes showed different banding patterns in control and irradiated plants (Figs. 1a-c). The level of polymorphism was higher in gamma rays treated plants than control plants. The extra alleles in treated plants could be the result of changes (mutations) at molecular level. Our findings are in line with those of Fadia *et al.,* (2011), who recorded maximum genetic variability in gamma rays treated genotypes of *Hibiscus subdariffa*by using RAPD primers. The genetic distances (GD) values varied among all tested genotypes (Table 2; Fig. 2). Our finding showed similarities with those of *Hamideldin* and *Eliwa* (2015), who reported that gamma radiation treatments with seeds of *Brassica alba* L. cause both physiological and molecular changes. Morita *et al.,* (2009) found deletion, substitution and inversion mutation in rice genotypes treated with gamma rays.

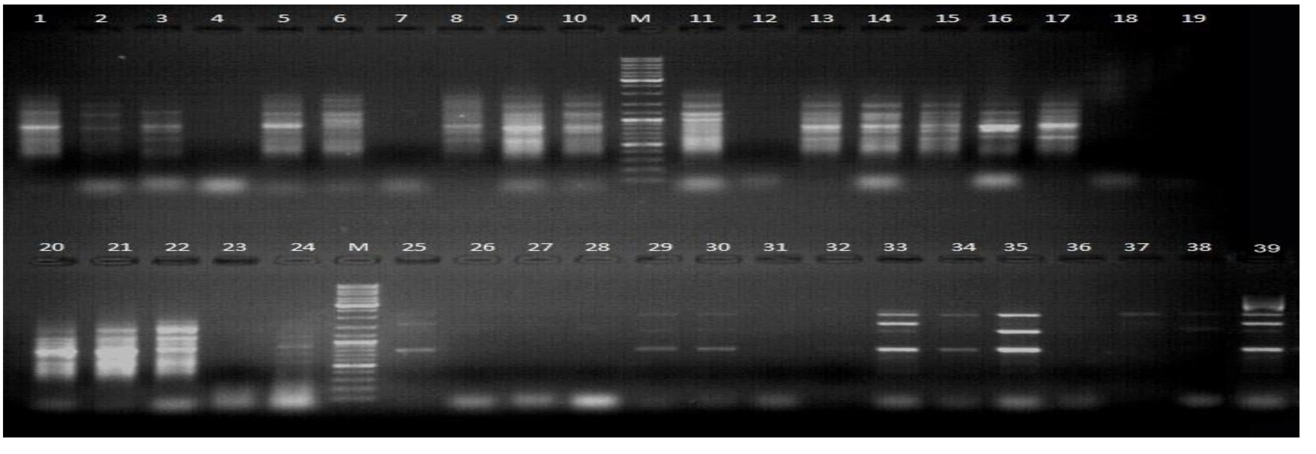

Fig. 1a. PCR amplification profile of thirty nine tea plants using RAPD primer (AC–05) M = molecular size marker (1 kbp). 1, 2, 3 = control, 4 – 39 = treated.

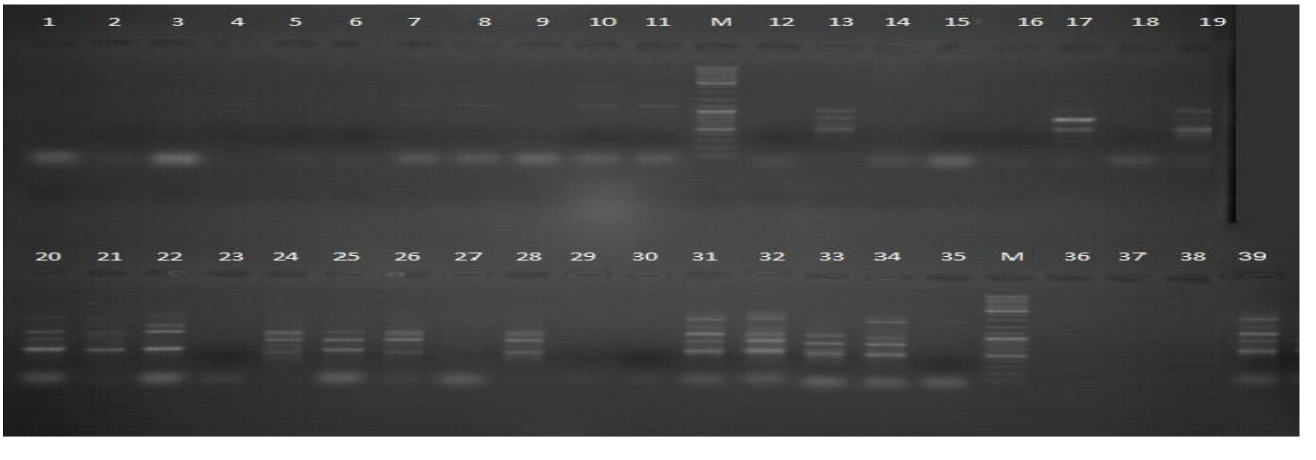

Fig. 1b. PCR amplification profile of thirty nine tea plants using RAPD primer (AC–09). M = molecular size marker (1 kbp). 1, 2, 3 = control, 4 – 39 = treated.

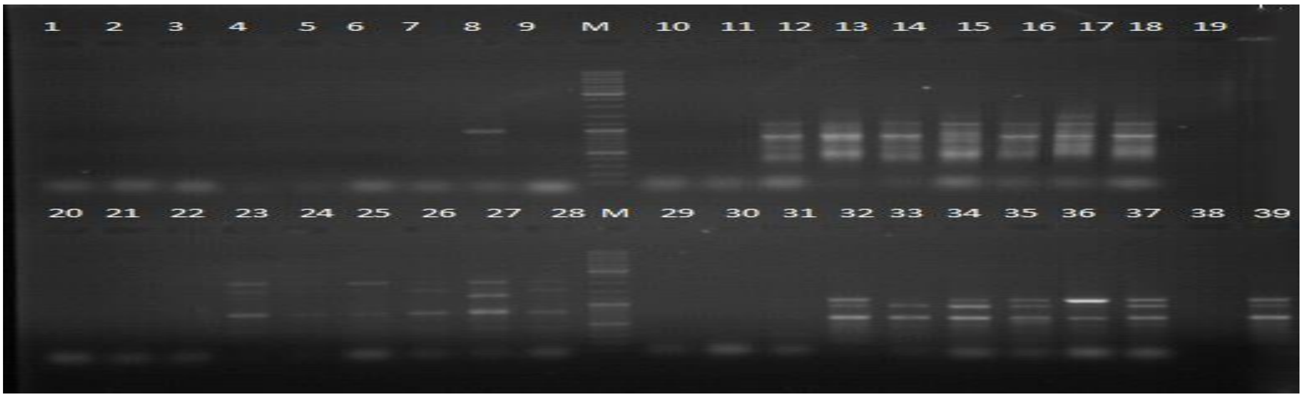

Fig. 1c. PCR amplification profile of thirty nine tea plants using RAPD primer (OPB -1). M = molecular size marker (1 kbp). 1, 2, 3 = control, 4 – 39 = treated.

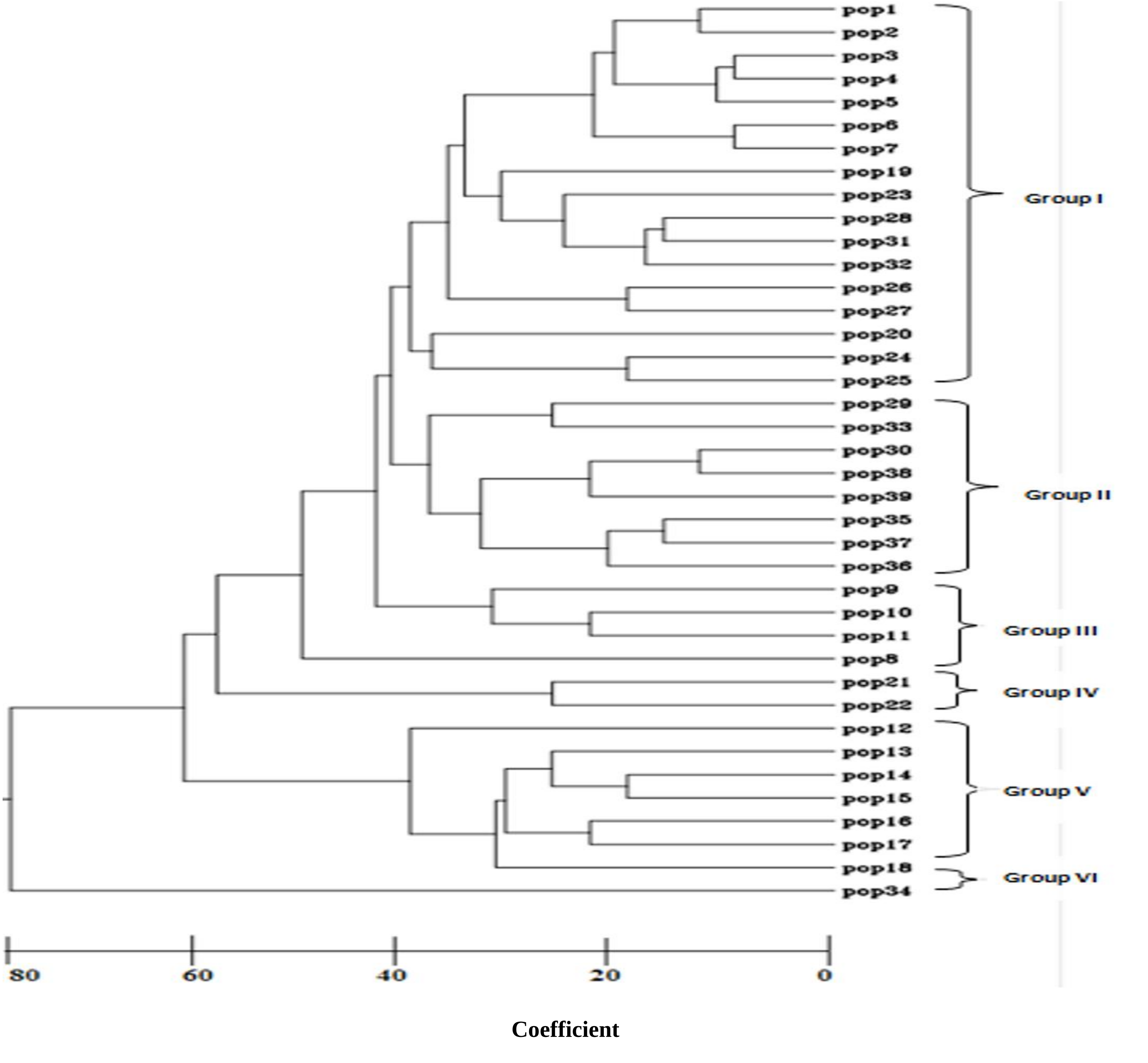

Fig. 2. Dendrogram constructed for thirty nine tea genotypes by using RAPD primers.
Pop1 = Qi-men, pop 2 = P-3, pop 3 = Indonesian, pop 4 = M1, pop 5 = M2, pop 6 = M3, pop 7 = M4, pop 8 = M5, pop 9 = M6, pop 10 = M7, pop 11= M8, pop 12 = M9, pop 13= M10, pop 14 = M11, pop 15 = M12, pop 16 = M13, pop 17 = M14, pop 18 = M15, pop 19 = M16, pop 20 = M17, pop 21 = M18, pop 22 = M19, pop 23 = M20, pop 24 = M21, pop 25 = M22, pop 26 = M23, pop 27 = M24, pop 28 = M25, pop 29 = M26, pop 30 = M27, pop 31 = M28, pop 32 = M29, pop 33 = M30, pop 34 = M31, pop 35 = M32, pop 36 = M33, pop 37 = M34, pop 3 8 = M35, pop 39 = M36.

Table 2. DICE similarity matrix among 39 tea genotypes using RAPD primers.

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.57	1.00													
3	0.33	0.29	1.00												
4	0.25	0.67	0.75	1.00											
5	0.29	0.50	0.57	0.67	1.00										
6	0.40	0.33	0.02	0.02	0.00	1.00									
7	0.40	0.33	0.00	0.00	0.11	0.50	1.00								
8	0.10	0.10	0.10	0.12	0.00	0.22	0.22	1.00							
9	0.40	0.18	0.20	0.17	0.18	0.00	0.00	0.14	1.00						
10	0.20	0.18	0.10	0.17	0.18	0.00	0.22	0.29	0.57	1.00					
11	0.40	0.18	0.00	0.00	0.10	0.44	0.44	0.29	0.43	0.57	1.00				
12	0.20	0.18	0.40	0.33	0.18	0.10	0.00	0.29	0.29	0.14	0.29	1.00			
13	0.22	0.21	0.22	0.30	0.21	0.12	0.10	0.18	0.36	0.27	0.27	0.45	1.00		
14	0.36	0.17	0.36	0.31	0.33	0.00	0.11	0.13	0.53	0.40	0.40	0.53	0.70	1.00	
15	0.00	0.11	0.18	0.15	0.17	0.00	0.00	0.27	0.13	0.13	0.13	0.53	0.61	0.63	1.00
16	0.25	0.44	0.25	0.40	0.22	0.00	0.11	0.33	0.33	0.00	0.13	0.33	0.40	0.46	0.31
17	0.13	0.25	0.27	0.35	0.25	0.14	0.00	0.42	0.21	0.00	0.21	0.53	0.67	0.50	0.60
18	0.00	0.20	0.12	0.18	0.00	0.12	0.00	0.31	0.31	0.15	0.15	0.46	0.48	0.43	0.57
19	0.00	0.22	0.00	0.20	0.10	0.00	0.29	0.33	0.00	0.17	0.17	0.17	0.40	0.00	0.15
20	0.17	0.15	0.17	0.14	0.15	0.18	0.18	0.50	0.25	0.13	0.25	0.13	0.17	0.12	0.00
21	0.13	0.00	0.13	0.11	0.12	0.13	0.00	0.40	0.40	0.30	0.50	0.30	0.43	0.38	0.29
22	0.13	0.00	0.13	0.11	0.12	0.13	0.00	0.30	0.40	0.30	0.50	0.30	0.43	0.38	0.29
23	0.00	0.12	0.00	0.00	0.11	0.00	0.00	0.00	0.10	0.00	0.20	0.20	0.11	0.18	0.18
24	0.44	0.20	0.22	0.18	0.40	0.25	0.25	0.31	0.31	0.31	0.46	0.15	0.29	0.29	0.00
25	0.25	0.22	0.25	0.20	0.22	0.00	0.29	0.17	0.33	0.33	0.50	0.33	0.30	0.31	0.15
26	0.13	0.11	0.00	0.10	0.00	0.11	0.33	0.18	0.00	0.18	0.18	0.12	0.11	0.00	0.00
27	0.11	0.00	0.11	0.15	0.12	0.00	0.11	0.00	0.11	0.13	0.00	0.00	0.11	0.00	0.12
28	0.00	0.12	0.12	0.14	0.00	0.00	0.40	0.20	0.00	0.20	0.20	0.11	0.11	0.00	0.00
29	0.22	0.00	0.00	0.10	0.00	0.12	0.00	0.15	0.31	0.31	0.31	0.00	0.10	0.14	0.00
30	0.44	0.20	0.00	0.12	0.00	0.25	0.25	0.15	0.15	0.15	0.31	0.00	0.10	0.14	0.00
31	0.25	0.22	0.10	0.00	0.00	0.29	0.29	0.10	0.00	0.00	0.17	0.00	0.20	0.00	0.00
32	0.00	0.00	0.12	0.00	0.10	0.11	0.00	0.00	0.11	0.00	0.00	0.00	0.20	0.00	0.00
33	0.22	0.00	0.00	0.11	0.00	0.00	0.00	0.15	0.31	0.31	0.31	0.00	0.19	0.14	0.00
34	0.00	0.13	0.00	0.12	0.13	0.00	0.14	0.21	0.00	0.21	0.21	0.11	0.30	0.10	0.10
35	0.25	0.12	0.00	0.00	0.10	0.00	0.00	0.17	0.17	0.17	0.33	0.17	0.20	0.31	0.15
36	0.00	0.00	0.40	0.29	0.00	0.00	0.00	0.12	0.00	0.11	0.22	0.44	0.12	0.20	0.20
37	0.00	0.00	0.11	0.00	0.14	0.00	0.12	0.15	0.00	0.10	0.13	0.00	0.00	0.00	0.00
38	0.29	0.10	0.11	0.00	0.10	0.11	0.10	0.00	0.18	0.18	0.36	0.18	0.21	0.33	0.17
39	0.22	0.00	0.22	0.18	0.10	0.00	0.00	0.15	0.15	0.15	0.31	0.31	0.19	0.29	0.14

Table 2. (Cont'd.).

Genotype	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
16	1.00																							
17	0.59	1.00																						
18	0.36	0.44	1.00																					
19	0.20	0.35	0.36	1.00																				
20	0.29	0.38	0.00	0.29	1.00																			
21	0.11	0.48	0.21	0.22	0.55	1.00																		
22	0.11	0.48	0.21	0.22	0.45	0.77	1.00																	
23	0.10	0.13	0.22	0.00	0.17	0.25	0.38	1.00																
24	0.18	0.33	0.00	0.36	0.53	0.32	0.32	0.00	1.00															
25	0.20	0.35	0.18	0.40	0.43	0.33	0.33	0.25	0.55	1.00														
26	0.00	0.13	0.00	0.44	0.31	0.12	0.24	0.29	0.40	0.44	1.00													
27	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	1.00												
28	0.10	0.13	0.10	0.50	0.50	0.25	0.25	0.33	0.44	0.50	0.57	0.00	1.00											
29	0.00	0.00	0.00	0.00	0.27	0.42	0.32	0.22	0.17	0.18	0.00	0.25	0.22	1.00										
30	0.00	0.00	0.00	0.00	0.27	0.32	0.32	0.44	0.17	0.00	0.20	0.00	0.22	0.50	1.00									
31	0.10	0.12	0.00	0.40	0.43	0.33	0.22	0.25	0.18	0.20	0.22	0.00	0.50	0.18	0.36	1.00								
32	0.00	0.12	0.00	0.40	0.43	0.33	0.33	0.25	0.18	0.20	0.22	0.00	0.50	0.18	0.18	0.80	1.00							
33	0.10	0.11	0.00	0.18	0.40	0.53	0.42	0.22	0.33	0.36	0.20	0.10	0.44	0.67	0.67	0.36	0.36	1.00						
34	0.00	0.17	0.11	0.35	0.48	0.48	0.32	0.40	0.22	0.35	0.38	0.10	0.40	0.22	0.44	0.47	0.47	0.44	1.00					
35	0.00	0.12	0.18	0.00	0.14	0.33	0.33	0.50	0.18	0.20	0.22	0.10	0.00	0.36	0.73	0.00	0.00	0.55	0.47	1.00				
36	0.10	0.14	0.25	0.00	0.00	0.13	0.13	0.40	0.00	0.29	0.00	0.10	0.00	0.11	0.00	0.00	0.00	0.00	0.14	0.29	1.00			
37	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.29	0.00	0.00	0.29	0.15	0.33	0.00	1.00		
38	0.00	0.13	0.20	0.00	0.00	0.24	0.35	0.57	0.20	0.22	0.25	0.00	0.00	0.20	0.60	0.00	0.00	0.40	0.38	0.82	0.33	0.40	1.00	
39	0.00	0.11	0.17	0.00	0.13	0.32	0.32	0.44	0.17	0.18	0.20	0.00	0.00	0.33	0.67	0.00	0.00	0.50	0.44	0.81	0.50	0.29	0.80	1.00

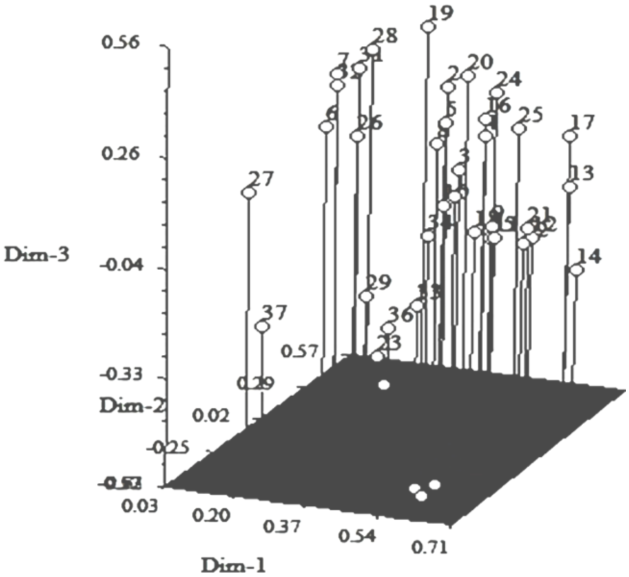

Fig. 3. 3D structure analysis of 39 Tea genotypes irradiated with gamma rays.

The phylogenetic analysis classified all genotypes into 6 major groups (I-VI). The group I was the largest group consisted 17 genotypes followed by 8 genotypes in group II. The group III-VI included 4, 2, 7, and 1 genotype, respectively (Fig. 2). Among the studied genotypes, the Qi–men, M19 and M31 were highly diverged types and these genotypes are recommended for further molecular analysis (Fig. 2). These findings were further confirmed through Principal Coordinate Analysis (PCoA) by using DICE similarity coefficient values. The 3D structure showed that genotypes Qi men, M19, M28, and M31 are highly diverged from rest ones (Fig. 3). Wang *et al.,* (2017) recorded Jaccard's coefficients of dissimilarity values of 0.6885 to 1.000 in *Sophoradavidii* (Franch.) Kom. ex Pavol genotypes treated with 20–140 Kr gamma rays though inter-simple sequence repeat (ISSR) markers. In addition they noted five diverged mutants groups through phylogenetic analysis. Jan *et al.,* (2019) also identified elite genotypes of Guar through PCoA method. The present study serves as a model to bring novel mutation in other local and exotic tea genotypes through radiation therapy.

Conclusion

The 16 extra alleles were recorded in 39 gamma irradiated tested experimental tea materials with 8 RAPD markers. The genetic distances values varied among tested genotypes. High level of polymorphism was recorded among genotypes. The presence of extra alleles in these genotypes shows that current diverged genotypes show similarity with previously studied novel tea genotypes *i.e.* Qi men, P3 and Indonesian. This similarity with previously approved cultivars might be a result of mutations that might have occurred by gamma radiations. The low dose (10 Kr) of gamma radiations is highly recommended to bring novel variations in other tea genotypes.

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