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Effect of gamma radiation on in vitro morphogenesis, anatomy and DNA polymorphism of *Moringa concanensis* Nimmo

Nikita Gautam, Priyanka Faroda, Shivani Shekhawat & Amit Kumar Gupta✉

The current study explores the impact of gamma radiation on the in vitro morphogenesis of *Moringa concanensis*. In vitro regenerated shoots were exposed to ^{133}Barium and ^{57}Cobalt gamma radiation sources for varying lengths of time (3, 6, 9, and 15 min). All the treated shoots survived with 100% regeneration frequency. The number of regenerated shoots was increased to 4.33 ± 1.57/inoculum in cobalt radiation-treated shoots. The field survival rate was increased, and 70% of plantlets from gamma radiation-treated shoots were successfully transferred to polybags. The multiple layers of epidermis, elongated cortical cells, pericycle cells, and increased content of vascular elements were observed in the anatomical assessment of regenerated shoots after treatment. The variations and altered responses of the treated shoots were further evaluated through CBDP (CAAT Box Derived Polymorphism), SCoT (Start Codon Targeted) gene-based, and ISSR (Inter Simple Sequence Repeat) intergenic sequence-based markers. An effective range of polymorphism of 75.00%, 77.77%, and 80.76% was observed from all the employed primers. A total of 0.2 PIC value was obtained from all used 6 primers that represent their informativeness in evaluating diversity among genotypes. The given minimum dose influenced the in vitro growth, anatomical development, and variations in genomic sequences, proving gamma radiation as an effective mutagen for *Moringa concanensis*. The gamma radiation source ^{133}Ba and ^{57}Co would be further used as a physical mutagen for developing efficient varieties of *Moringa concanensis* for the *Moringa* breeding program.

Keywords Physical mutagen, Point mutation, Molecular markers, CBDP, SCoT, ISSR

Abbreviations

BAP	6-Benzyl amino purine
CBDP	CAAT box derived polymorphism;
CTAB	Cetyltrimethylammonium bromide
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
ISSR	Inter simple sequence repeat
MMS	Modified murashige and skoog
PGRs	Plant growth regulators
SCoT	Start codon targeted

Mutations are the altered genotypic structure of individuals that could be responsible for their adaptations to the environment and genetic improvement[1]. Gamma radiation (ionizing radiation) has been widely used as a mutagen for mutation and variation breeding for plant improvement programs in the last few decades. These rays are more feasible than other ionizing irradiations due to their cost-effectiveness, less damage, and high penetration power to tissues[2]. It can either directly alter the genetic structure or indirectly induce reactive oxygen species that ultimately create DNA damage[3]. Many reports revealed that the effects of gamma irradiation are dose-dependent; lower exposure can stimulate self-defense, cell proliferation, abiotic stress resistance, enzyme activity, seed germination, and crop yields[2], while higher exposure can disrupt many functions, including biochemical and physiological processes, through DNA damage[4]. The analysis of anatomical changes in gamma

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radiation-influenced shoots can aid in the understanding of the alteration in tissue content and arrangement of the cells[5].

Advancements and evolution in molecular marker techniques have simplified the detection of changes in the DNA profile of an individual[4]. CBDP and SCoT are efficient, reproducible, informative gene-targeted markers that evaluate polymorphism among coding sequences. SCoT is a start codon-targeted marker based on the flanking region of the ATG codon[6]. CBDP is distinctly located ~80 bp upstream of the start codon, based on the promoter region of plant gene[7]. Both of these markers are efficiently used for polymorphism assessment for various species like *Aegilops*[8], *Calligonum polygonoides*[9], *Centaurea* spp[10], *Moringa concanensis*[11], *Simmondsia chinensis*[12], and *Ziziphus mauritiana*[13]. Besides this, the ISSR marker has been utilized to amplify the intergenic sequences for genetic diversity assessment among *Moringa* individuals[14–17]. The combinations of these markers can evaluate the whole introduced variation in the genome by mutagens.

Moringa concanensis Nimmo is a wild relative species of *Moringa oleifera* belonging to the monogeneric family Moringaceae. It is also an endemic species of the Indian sub-continent[18]. It is a good source of vitamins A and C, antioxidants, non-desiccating oil (Ben oil), and traditional medicines[18–20]. As a potential species, it can be used to transfer traits to cultivated species, *Moringa oleifera*[21]. Olson et al.[22] earlier suggested that the hybrid of *Moringa concanensis* and *Moringa oleifera* has maximum nutritional values than all 13 *Moringa* species of the Moringaceae family. Therefore, the present investigation aims to improve *Moringa concanensis* through gamma radiation-induced in vitro mutagenesis. Further, the DNA polymorphism analysis was performed to validate the introduced variation in the genomic content of treated shoots. This present communication is the first report on gamma radiation-induced in vitro morphogenesis of *M. concanensis*, using ^{57}Co and ^{133}Ba, and applying anatomical and DNA polymorphism analysis with CBDP, SCoT, and ISSR dominant markers.

Materials and methods

Plant material and irradiation procedure

Stock cultures (Fig. 1A) of in vitro regenerated shoots of *Moringa concanensis*[23] were used as a source of plant material. Shoots with shoot tips ranging 2–4 cm in length were excised and first transferred to culture tubes containing solidified agar devoid of any nutrients and plant growth regulators. The plant sample used for establishing the stock culture was initially identified by submitting a herbarium sheet to the Botanical Survey of India, Jodhpur, Rajasthan, approved identification number: BSI/AZRC/I.12012/Tech./2022-23 (PI.-Id)/36. Cultured tubes were exposed to Gamma irradiation sources (^{133}Ba 80.99 keV and ^{57}Co 122 keV) individually. The shoots were exposed to radiation sources at different 3, 6, 9, and 15-min intervals. The distance between the source and culture tube was kept constant at 1 cm, and the irradiation process was conducted at the Department of Physics, Mohanlal Sukhadia University, Udaipur, in the dark box under expert supervision. The range of absorbed dose was 0.1 Gy, 0.2 Gy, 0.3 Gy, 0.5 Gy from ^{133}Ba and 0.04 Gy, 0.08 Gy, 0.12 Gy and 0.2 Gy ^{57}Co sources, measured using a dosimeter.

Determination of in vitro growth

After exposure to gamma radiation, the shoots were transferred to modified MS media[24] containing 0.5 mg/L BAP and 0.1 mg/L IAA. The cultures were incubated for further growth under-maintained conditions as described by Gautam et al.[23]. In vitro growth was observed after 10–15 days of inoculation in terms of survival efficiency, shoot regeneration, shoot number, shoot length, and the appearance of regenerating shoots. In vitro regenerated shoots without irradiation treatment were used as controls. After that, regenerated shoots were further subjected to an in vitro rooting and hardening process as previously described in our report, Gautam et al.[23]. The successfully hardened plantlets were transferred to polybags.

Determination of anatomy

The tissue arrangement of the control and regenerated shoots after treatment was observed to evaluate the effect of gamma radiation on the anatomy of the regenerated shoots. Transverse sections from treated and control shoots were observed under a compound light microscope (Carl Zeiss). The specimens were prepared following Gautam et al.[23].

Determination of DNA polymorphism

The genetic variations introduced by gamma radiation in the developed shoots were assessed using SCoT, CBDP, and ISSR primers. Genomic DNA of in vitro regenerated shoots from treated explants and the mother plant (control) was isolated using the CTAP method [25,26]. Two primers from each were selected after screening five primers of SCoT, CBDP, and ISSR (Table 2). The PCR reaction (25 µl) mixtures for selected primers were prepared using 2.5 µl of 10X PCR buffer (100 mM Tris, pH 9.0, 500 mM KCl), 1.5 µl of 25 mM $MgCl_2$, 1.0 µl of 2.5 mM dNTPs, 1.5 µl of 0.6 mM each of primer, 1.5 µl of 1 U/µl Taq DNA polymerase, 13.0 µl sterile nuclease-free water, and 4 µl of 40 ng template DNA. The PCR reactions were carried out for 40 cycles containing 5 min of denaturation at 94 °C, 1 min of annealing at 50.3 °C for SCoT, 50 °C for CBDP, and 44 °C for ISSR, 1 min of elongation at 72 °C, and final extension for 10 min at 72 °C. The amplicons were visualized under a gel documentation system after being separated in agarose gel electrophoresis at 80 V and 120 A for 2 h in TBE buffer (pH 8.0).

Statistical analysis

Experiments were set as per the randomized block design. Each in vitro experiment of irradiation treatment was repeated three times with six replicates per treatment in each experiment. Data were recorded at 10–15 days of time intervals. The results are expressed as mean ± SD of three experiments and were subjected to one-way analysis of variance (ANOVA) with mean separation ($P \leq 0.05$) by Duncan's multiple range test. Statistical analysis

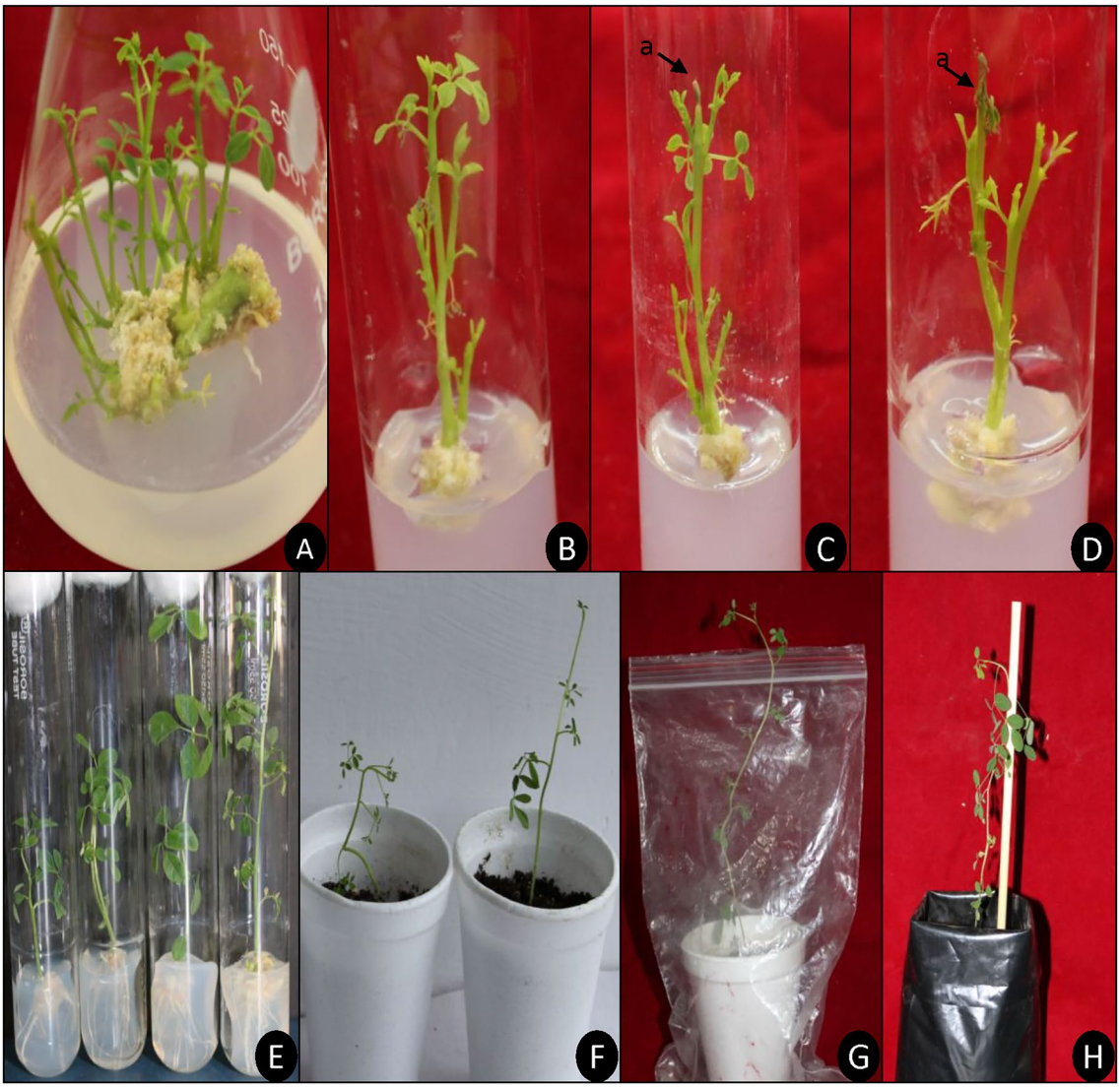

Fig. 1. Effect of gamma radiation on in vitro morphogenesis of *Moringa concanensis*: (**A**) In vitro culture of *Moringa concanensis*; (**B**) Untreated shoots; (**C**) Cobalt radiation-treated shoot; (**D**) Barium radiation-treated shoot; (**E**) In vitro rooting; (**F–G**) Plantlets under hardening phase; (**H**) Plantlet in polybag.

was done using SPSS v.27 (IBM-SPSS). Other assessments, including anatomy and polymorphism, were also repeated three times with three replicates. Each PCR-amplified SCoT, CBDP, and ISSR fragments were scored as binary data, and the Polymorphic Information Content (PIC), Resolving Power (RP), Effective Multiplex Ratio (EMR), and Marker Index (MI) for all utilized markers were calculated according to Faroda et al.[26]. The heat map was constructed using the binary data of primers obtained from the studied mutants and the control sample. Genomic template stability was calculated using the $GTS = (1 - a/n) \times 100$ formula, where a represent number of polymorphic and n was the number of total bands[27]. FreeTree program version 0.9.1.5[28] and FigTree version 1.2.2. were used to cluster analysis and construct a dendrogram after calculating a pair-wise matrix of genotype distances using the Jaccard coefficient and permitting a 1000-iteration bootstrap test with the same algorithm.

Results and discussion

Effect of gamma radiation on in vitro propagation

The type of radiation source and exposure duration affected the in vitro morphogenesis of the *Moringa concanensis*. The present study found no detrimental effect on the bud-breaking response of treated in vitro shoots. Inoculated shoots from each type of exposure survived and responded with a maximum regeneration frequency of 100%, similar to the control (Fig. 1B). However, a previous report on *Rosa hybrida*[29] found a decreasing survival rate of treated explants. The authors reported that a higher intensity of exposure impaired the explant's response in in vitro conditions. We observed burned shoot tips of the treated shoots, but it didn't affect the regeneration response of the shoots to nutritional media (Fig. 1Ca & Da). Both applied radiation sources slightly affected the number of in vitro regenerated shoots, while observed as insignificant for the shoot length as

well. Meanwhile, the cobalt source was effective for the bud breaking with the highest 4.33 ± 1.57 shoot number/inoculum (Table 1, Fig. 1C). The efficiency of the cobalt radiation source was earlier reported by Aly et al.[30] on *Rubus fruticosus* and Ghani and Sharma[31] on *Gerbera jamesonii.* Aly et al.[30] also reported the dose-dependent inhibition of the growth in micropropagation of *Rubus fruticosus*. Here, the exposure slightly enhanced the number of regenerating shoots from the control inoculum. May, the effect was also dose-dependent; it decreased to 4.11 ± 2.08/inoculum with increasing the duration of gamma exposure to 0.2 Gy (Table 1). The barium source was not efficient in inducing bud break. The reported highest shoot number from barium-treated shoots was fewer than the control. It was observed that only 3.38 ± 1.41 shoots/inoculum (Table 1, Fig. 1D) regenerated at the shortest 3-min/0.1 Gy exposure duration. The effect of radiation exposure would be due to the crosstalk between the radiation and internal hormonal metabolism[29]. The imbalance of hormone metabolism directly affects the rate of in vitro regeneration.

After the regeneration of shoots, a maximum of 100% (Fig. 1E) in vitro rooting was attained on IBA 1.0 mg/l containing 1/4th MMS media as previously described in Gautam et al.[23]. The process for hardening and acclimatization was also the same as our previous study. However, the field survival of the gamma radiation-treated plantlets was increased up to 70% (Fig. 1F, G, H) from the previously mentioned 60% rate in our study[23]. The anatomical assessment of the in vitro regenerated shoots was done to evaluate the reason behind this.

Effect on in vitro anatomical development

The treatment of the gamma source affected the tissue content (quantity and quality of cells) of the plants[32]. The regenerated shoots were observed and compared to the mother cultures of *Moringa concanensis*. The arrangement of tissue was found equivalent in both mother and treated shoots in the transverse sections (Fig. 2A, C). Both the radiation sources, ^{133}Ba and ^{57}Co, equally affected the regenerating shoots. It initiated with the cuticular epidermis (Fig. 2A, C) from the outer sides and ended with the pith (Fig. 2A, B). The arrangement of the cortex, pericycle, and vascular bundle was also well-mannered. The exposure to gamma radiation increased the number of epidermal layers in the developing shoots (Fig. 2C). The cells of the cortex were differentiated into two types, one was compactly arranged and the second was loosely present with a hypertrophied and elongated appearance (Fig. 2D). The hypertrophied cells were also present in mother shoots, but were shorter in size compared to the treated shoots. The cortical parenchymatous cells were extra-long, unorganized, loosely packed, and invaded into vascular tissue (Fig. 2D), divergent from mother shoots. It may be due to the surface or localized penetration of gamma rays from the sources, which influences the elongation or loose packing of cells by affecting the cell wall composition and decomposition of the middle lamella[33]. The layer of pericycle was observed in discontinuous patches covering the individual vascular bundle (Fig. 2E). However, the cells were sclerenchymatous but found elongated after radiation treatment. Similar results of elongation of cells were found in *Graptophyllum pictum* leaf tissue after gamma radiation treatment[5]. The xylem and phloem elements were increased in treated shoots, independent of the duration of radiation exposure (Fig. 2F). It was earlier reported that gamma radiation affected and enhanced the vascular elements of the stem[34]. The presence of mechanical tissues supports in vitro-*raised* plantlets to survive under acclimatization and field conditions[35,36]. The multilayered epidermis and higher vascular elements would be a reason behind the increased survivability of plantlets, for the higher survivability obtained in the present study.

Effect on DNA polymorphism

Three molecular makers, SCoT, CBDP, and ISSR, were used to assess the introduced genomic variations in treated shoots (Fig. 3). These combinations of markers evaluated the variations in both genes (CBDP and SCoT) and intergenic (ISSR) sequences. The CBDP and SCoT markers have been proven efficient in assessing variation in gene sequences[8,10]. The reproducibility of ISSR in evaluating polymorphism among the *Moringa* genome is also well-known[14]. Five primers from each marker were screened to evaluate the polymorphism. Two primers from each screened primer were selected for further analysis based on their reproducibility and scoring. A total of 77.77%, 80.76%, and 75% polymorphism from SCoT, ISSR, and CBDP primers was obtained (Table 2).

S. No	Radiation source	Radiation duration	Response (%)	Shoot number/explant	Shoot length (cm)
1		Control	100	4.16 ± 0.76^{ab}	2.46 ± 0.62^{a}
2	Barium (^{133}Ba)	3 min	100	3.38 ± 1.41^{abc}	2.21 ± 0.89^{ab}
3		6 min	100	3.00 ± 1.36^{bc}	2.45 ± 1.09^{a}
4		9 min	100	2.46 ± 1.35^{c}	2.14 ± 0.66^{ab}
5		15 min	100	3.22 ± 1.00^{abc}	1.66 ± 0.43^{b}
6	Cobalt (^{57}Co)	3 min	100	3.55 ± 0.98^{ab}	2.08 ± 0.64^{ab}
7		6 min	100	4.05 ± 1.73^{ab}	2.15 ± 0.98^{ab}
8		9 min	100	4.33 ± 1.57^{a}	2.08 ± 1.12^{ab}
9		15 min	100	4.11 ± 2.08^{ab}	2.19 ± 0.72^{ab}

Table 1. Effect of gamma radiation on shoot regeneration of *Moringa concanensis* (MMS + 0.5 mg/L BAP + 0.1 mg/L IAA). Each experiment was repeated thrice with six replicates per treatment. Data were regularly recorded after intervals of 10–15 days. Different lowercase letters based on Duncan's test at $P \leq 0.05$ indicate significant differences among treatments by one-way ANOVA. BAP, 6-Benzyl Amino Purine; IAA, Indole-3-acetic Acid MMS; Modified Murashige and Skoog.

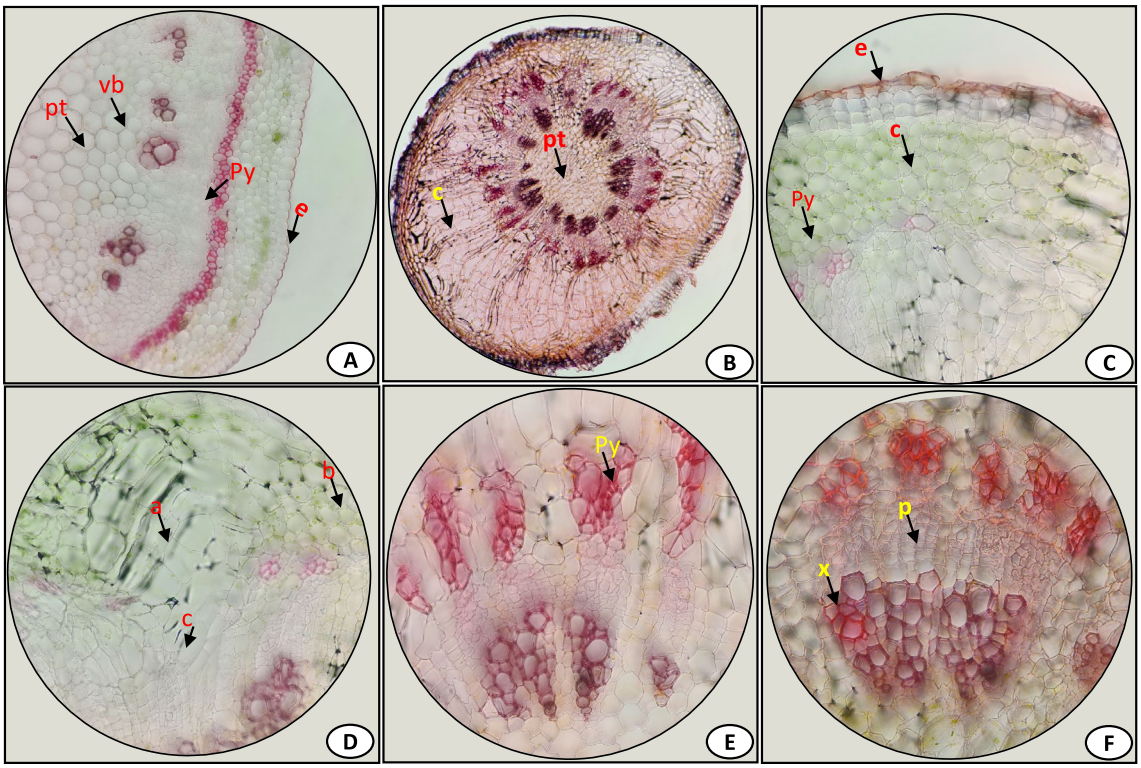

Fig. 2. In vitro anatomy of gamma radiation-treated shoots: (**A**) T.S. of untreated shoot; (**B–F**) T.S. of treated shoot; (**B**) Whole section of shoot; (**C**) Multiple epidermis layers; (**D**) Differentiated and invaded cortex tissue; (**E**) Elongated and discontinuous pericycle; (**F**) Vascular tissue (e—epidermis, c—cortex, py—pericycle, vb—vascular bundle, pt—pith).

SCoT primers produced 27 amplicons with an average of 13.5 bands per primer (Fig. 3A, B). Among them, 21 (77.77%) bands were observed to be polymorphic with an average of 10.5 polymorphic bands per primer. SCoT 3 was more polymorphic with 12 polymorphic bands and 80% polymorphism (Table 2, Fig. 3A). CBDP primer produced 20 amplicons, of which 15 (75%) were polymorphic (Table 2, Fig. 3C, D). These primers had an average of 10 bands per primer, and 7.5 bands were polymorphic. CBDP 13 was more informative with 12 polymorphic bands and 85.71% polymorphism (Table 2, Fig. 3D). While CBDP 9 had fewer polymorphic bands (03) and polymorphism (50%) among all used primers (Table 2, Fig. 3C). A total of 26 amplicons with an average of 13 bands per primer were obtained from employed ISSR primers (Table 2, Fig. 3E, F). Among them, 21 (80.76%) bands were polymorphic with an average of 10.5 polymorphic bands per primer. ISSR (UBC 11) was more informative with a total of 12 polymorphic bands and 85.71% polymorphism.

The highest number of amplicons (27) was observed from SCoT primers, while the highest polymorphic amplicons (21/26) with higher polymorphism (80.76%) were from ISSR primers. However, individually, CBDP 13 and ISSR UBC 11 primers exhibited the highest polymorphic bands with higher polymorphism. The results are similar to the previous findings of Atia et al.[10] on *Centaurea* species and Bokaei et al.[8] on *Aegilops triuncialis*, where the reproducibility of SCoT markers was higher than CBDP markers in the genetic diversity assessment. The obtained PIC value for all three targeted markers, SCoT 0.283, ISSR 0.241, and CBDP 0.234, lies between the optimum range of PIC value for a primer to assess the diversity among genotypes[37]. Further, the other marker informative index values like Rp (SCoT 0.718, ISSR 0.663 & CBDP 0.682) and MI (SCoT 3.025, 2.600, & 4.147) were also found satisfactory in evaluating the variation of genotypes. These findings support the effectiveness of CBDP, ISSR, and SCoT markers in assessing genetic diversity, corroborating the results reported by Khodaee et al.[38]. The obtained polymorphism in gene and non-genic sequences suggests that the gamma radiation introduced variation at the molecular level. The morphological, developmental, and anatomical variations in treated shoots are due to alterations in genomic content in the effect of gamma radiation.

Genomic template stability (GTS)

Genomic template stability provides a qualitative measurement for the modified amplification profile of primer[27]. Among all the treatments, the highest genomic stability was found in cobalt treatments, ranging between 40 and 55.0%. Barium treatments exhibited the lowest genomic stability, ranging from 17.5 to 50.0% GTS (Table 3). The range of newly appeared bands was found between 9 and 23 N, and approximately 15 bands disappeared after treatment. Individually, in 3 min of Ba treatment, 23 new bands appeared and 10 bands disappeared (Table 3, Fig. 4A). In contrast, the number of appeared and disappeared bands in Co 3 min treatments was 9 and 15, respectively (Fig. 4A). In 6 min of Ba and Co treatments, an equal number of 11 & 9 band appearance and

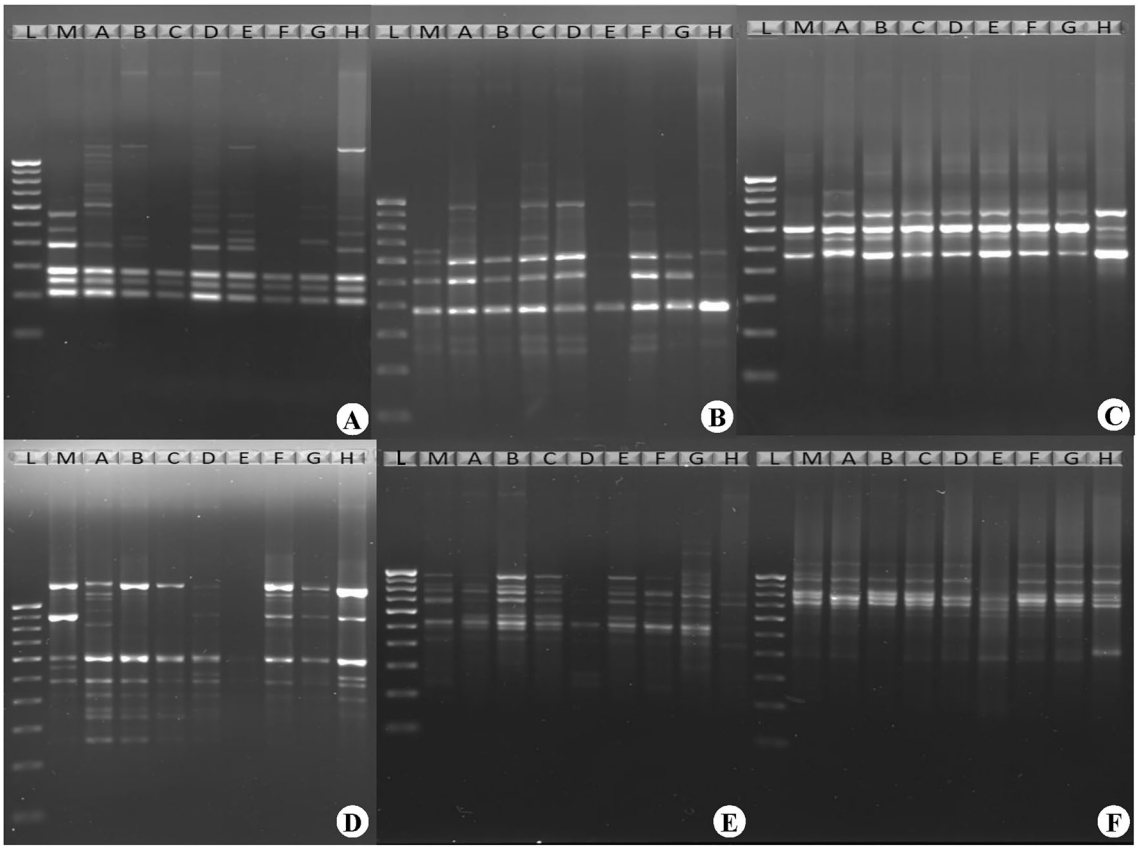

Fig. 3. DNA polymorphism analysis: (**A**) SCoT 3; (**B**) SCoT 15; (**C**) CBDP 9; (**D**) CBDP 13; (**E**) ISSR UBC 11; (**F**) ISSR UBC 18. (L—Ladder; M—Mother plant; A—3 min Barium treatment; B—6 min Barium treatment; C—9 min Barium treatment; D—15 min Barium treatment; E—3 min Cobalt treatment; F—6 min Cobalt treatment; G—9 min Cobalt treatment; H—15 min Cobalt treatment).

S.N.	Primer name	Primer sequence (5′-3′) (length)[a]	Tm °C	Ta °C	TB	MB	PB	% Polymorphism	PIC	RP	EMR	MI
SCoT MARKERS												
1.	SCoT -3	CAACAATGGCTACCACCG	53.90	50.30	15	3	12	80.00	0.316	0.751	12.99	3.792
2.	SCoT- 15	ACGACATGGCGACCGCGA	62.60	50.30	12	3	9	75.00	0.251	0.685	09.99	2.259
Total					27	6	21	77.77	0.283	0.718	11.49	3.025
ISSR MARKERS												
1.	UBC -811 (B)	GAGAGAGAGAGAGAGAC	46.80	44.30	14	2	12	85.71	0.282	0.698	11.99	3.386
2.	UBC -818 (D)	CACACACACACACACAG	51.00	44.30	12	3	9	75.00	0.201	0.629	10.99	1.814
Total					26	05	21	80.76	0.241	0.663	11.49	2.600
CBDP MARKERS												
1.	CAAT 9	TGAGCACGATCCAATGAT	51.20	50.00	06	03	03	50.00	0.148	0.611	03.99	0.444
2.	CAAT 13	TGAGCACGATCCAATGAC	52.00	50.00	14	02	12	85.71	0.320	0.753	12.99	3.851
Total					20	05	15	75.00	0.234	0.682	08.49	4.147

Table 2. Evaluation of DNA Polymorphism in gamma radiation-treated in vitro regenerated shoots of *Moringa concanensis*. Tm, melting temperature; Ta, annealing temperature; TB, total no of bands; MB, number of monomorphic bands; PB, number of polymorphic bands; PIC, Polymorphic Information Content; Rp, Resolving Power; EMR,Effective Multiplex Ratio; MI, Marker Index.

disappearances, with 45.0% and 55.0% GTS, was observed. Total of 40.0% & 52.5% GTS with 12 & 19 bands appearance and 12 & 9 band disappearance after 9 min treatment of BA and Co. The calculated total GTS for a 15-min exposure was 50.0% & 47.5%. A total of 11 & 9 bands appeared, and 9 & 12 bands disappeared after exposure (Table 3, Fig. 4). The obtained varying polymorphism among all gamma radiation-generated mutants had no relation to the applied dose. Similar results were also reported in *Triticum aestivum* mutants by Türkoğlu

S. No.	Radiation source	Radiation treatment (min)	Newly appeared disappeared band (N + D)	Total No of bands	Genetic template stability (GTS) (%)
1	Barium (^{133}Ba)	Control	–	40	–
2		3A	23N + 10D	51	17.5
3		6A	11N + 11D	40	45.0
4		9A	12N + 12D	40	40.0
5		15A	11N + 09D	42	50.0
6	Cobalt (^{57}Co)	3B	09N + 15D	34	40.0
7		6B	09N + 09D	40	55.0
8		9B	19N + 09D	40	52.5
9		15B	09N + 12D	37	47.5

Table 3. Effect of Gamma radiation on genomic template stability of in vitro regenerated shoot of *Moringa concanensis*.

et al.[27], where the mutagen dose did not affect the banding pattern. We have observed in our recently published study that the regenerated shoots were genetically stable, even after cold temperature treatment (94.44%)[11]. The altered banding patterns observed here, among the mutagen-treated samples, indicate the development of mutants in *Moringa concanensis*. The changes, including the appearance and disappearance of amplified bands, likely result from DNA methylation, DNA damage, and chromosomal abnormalities induced by the mutagenic treatment[39,40]. In addition, cluster analysis based on different primers yielded a similarity coefficient, which was used to construct a dendrogram. The samples could be classified into three distinct clusters, wherein all regenerated shoots after treatment may be considered mutants due to their low genetic similarity with the mother plant (Fig. 4B). These results are consistent with findings from gamma radiation-induced mutants of *Dendrobium*[41], *Vanilla planifolia*[42], and *Zamioculcas zamiifolia*[43].

Conclusion

The effect of gamma radiation, emitted from 133Ba and 57Co, as a mutagen on the in vitro regeneration of *Moringa concanensis* has been evaluated as a preliminary stage. The arisen mutation was found to be positive for plant growth, which increased the slight range of regenerated shoot number. It enhanced the field survival rate (70%) of in vitro plantlets, a crucial stage for micropropagated plantlets. The reason behind increased survivability may be the presence of increased mechanical tissues or vascular elements. Further, the dominant DNA markers CBDP, SCoT, and ISSR evaluated the range of mutagenesis-derived DNA polymorphism with a PIC value of 0.2. DNA polymorphism validated the introduced variation in genomic content, with the highest 80.76% polymorphism and three diverse groups from the control. This protocol would provide key primary information for future research with gamma radiation sources to develop an individual with an elite phenotype and a *Moringa* breeding program.

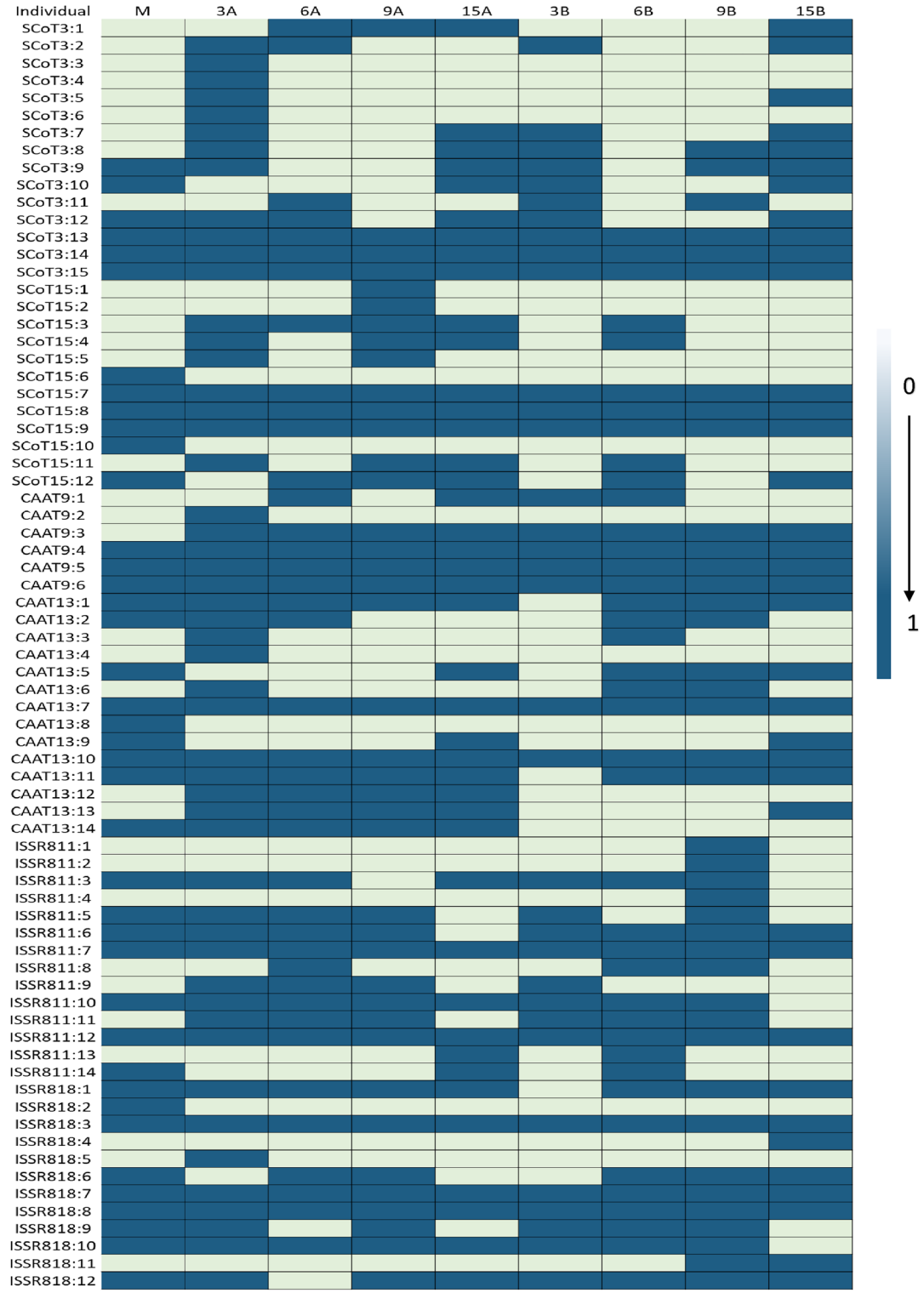

Fig. 4. (**A**) Heatmap of primer binary data (0 and 1) generated from SCoT, CBDP, and ISSR primers, untreated and after ^{133}Ba and ^{57}Co treatment of in vitro generated shoots; (**B**) Cluster analysis in vitro regenerated *Moringa concanensis* shoot (untreated and treated with mutagens). Genotypes: M—Mother plant; 3A—3 min Barium treatment; 6A—6 min Barium treatment; 9A—9 min Barium treatment; 15A—15 min Barium treatment; 3B—3 min Cobalt treatment; 6B—6 min Cobalt treatment; 9B—9 min Cobalt treatment; 15B—15 min Cobalt treatment.

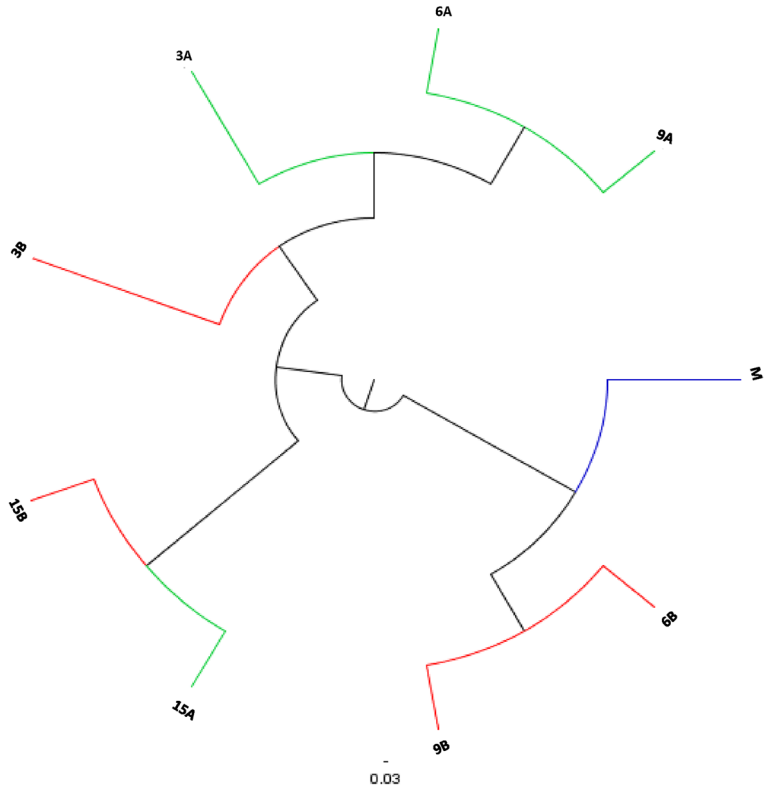

Fig. 4. (continued)

Data availability

The authors claim that all included data were acquired from the present research and have been indicated in the MS as well.

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Author contributions

A.K.G. and N.G. conceptualize the research. N.G. and S.S. conducted the experiments. N.G. and P.F. analyzed the data and drafted the manuscript. A.K.G. edited, revised, and prepared the final draft of MS. All authors approved the final manuscript.

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Declarations

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