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RESEARCH ARTICLE

DNA POLYMORPHISM AND MUTAGENESIS INDUCED IN OKRA (*ABELMOSCHUS ESCULENTUS* L.) EXPOSED TO PROJECTOR RADIATION USING SCOT MARKERS

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ABSTRACT

Okra (*Abelmoschus esculentus* L.) is one of the important crops grown mainly for its high nutritional and economic significance in the tropic and subtropic. The suitability of radiation from projector for inducing genetic diversity in okra was studied. Okra seeds were exposed to projected radiation at two distances from the projector (10 cm and 30 cm) for two durations (30 minutes and 1 hour), while seeds not exposed to radiations serves as controls for the study. The cetyltrimethylammonium bromide (CTAB) method was applied for the extraction of genomic DNA from young leaves of M1-generation plants while yield of DNA as well as the qualities were quantified using spectrophotometry. Similarly, the level of genetic variability was quantified using PCR amplification with three Start Codon Targeted (SCoT) markers to confirm the level of polymorphisms. Exposed okra seeds to radiation at a distance of 10 cm for 30 minutes significantly enhanced the DNA concentration, purity, and polymorphism, with the SCoT-1 primer detecting 100% polymorphism. When exposure duration was extended further to 1 hour, DNA degradation and reduction in the number of polymorphic bands were observed. Clustering followed genetic differentiation in relation to distance and duration of radiation exposure. The study indicates that projector radiation alters DNA concentration, purity, and create genetic variability in okra, with moderate doses optimizing genetic polymorphism while limiting degradation. These findings reveal that projector radiation is a potential cost-effective agent for generating genetic diversity during okra breeding programs.

KEYWORDS

Abelmoschus esculentus, Projector radiation, Genetic diversity, Start Codon Targeted (SCoT), DNA polymorphism.

1. INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) a vegetable extensively cultivated in the tropic and subtropics is treasured for its outstanding nutritional contents and economic importance. According to the study, okra contained optimum dietary fiber, valuable amino acids, and bioactive agents for human growth (Gaur et al., 2025). Nevertheless, as reported that narrow genetic base of the plant has limited its improvement which has affected the progress of traditional breeding in the crop (Behera et al., 2025; Singh and Pandey, 2024).

In this regard, increasing genetic variability through mutation breeding will help to development improved cultivars. Generally, physical mutagens such as gamma rays, and chemical mutagens like Ethyl Methane Sulphonate have been commonly used to induce variability in crops (Rana et al., 2024; Sawarkar and Mansoori, 2023). As reported that in recent times, radiation breeding has been valued for generating variabilities in crops because of the potentials to induce broad spectrum of genetic mutations at higher frequency which allows the development of novel traits with great precision (Bharat et al., 2024; Devi et al., 2024). Furthermore, the possibility to control dosage, adjust treatment durations, and reduce toxicity of physical mutagens compared to chemical mutagens makes the agent environmentally safer for crop improvement (Ma et al., 2021). However, among the physical mutagens, sources like X-rays, gamma rays, and fast neutrons raised safety concerns compared to others making them less suitable for modern crop improvement programs (Cardarelli, 2011).

Projector irradiation, a safer non-ionizing electromagnetic radiation with extremely low frequency waves has been reported to be a promising physical mutagen because of its affordability and accessibility (Lahir, 2023 and Usikalu et al., 2018). Despite these potentials, information is scanty on the genetic changes induced by projector radiation in crops which creates research gap on its mutagenic abilities. So far, studies have shown that projector radiation disrupts mitotic division and induce chromosome aberrations in *Allium cepa* and affects germination and growth parameters of okra (Alege et al., 2022a; Alege et al., 2022b). However, the molecular mechanisms guiding these cytological and morphological changes have not been properly investigated yet.

According to the study, quantifying the level of DNA polymorphism induced by a mutagen is fundamental to the success of any mutation breeding program (Yali and Mitiku, 2022). Start Codon Targeted (SCoT) markers are specifically suitable for this role because they target regions adjacent to the conserved ATG start codon which associate them with functional gene regions and ensures reproducibility of results according to (Rai, 2023). Further attributed the superiority of SCoT markers over other markers like RAPD, ISSR, SSR, and AFLP to high reproducibility potentials of the former, which increased their reliability for genetic

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analysis and mutation detection (Altaf et al., 2025). Moreover, highlighted that SCoT markers are cost-effective and do not require prior information on the genomic sequence which accounted for their wide usage (Igwe et al., 2022). The application of this sensitive and informative SCoT marker system will offer reliable methods for assessing functional genetic variations induced by mutagens for mutation breeding according to (Sankar et al., 2022).

Against this background, the study aims to assess the potentials of projector radiation for inducing DNA polymorphism and mutagenesis in okra using SCoT markers. The findings from the study are expected to complement the earlier reported effects of projector radiation on morphological attributes of okra and cytological characteristics of onion root tip cells (Alege et al., 2022b; Alege et al., 2022a). The findings will hopefully accelerate the process of developing improved okra cultivars through mutation breeding.

2. Materials and Methods

2.1 Plant Material and Radiation Treatment

Improved okra seeds (NH47-4) collected from the National Horticultural Research Institute (NIHORT) in Ibadan, Oyo State were cleaned and air-dried to a uniform moisture content before exposure to radiation from projector. For mutagenesis, the seeds were exposed to radiations from Dell 1210S projector manufactured by Dell Technologies in USA. This device has been confirmed to emit non-ionizing electromagnetic radiation at very low frequencies with color light wavelengths ranging from approximately 400 to 700 nm. The okra seeds were exposed continuously to projector radiation at distances of 10 cm and 30 cm from the projector lens for 30 minutes and 1 hour as suggested by (Alege et al., 2022a). Seeds not exposed to radiated served as the control for the study. To obtain M1 generation plants, post-irradiated and unirradiated seeds (control) were germinated and grown on Murashige and Skoog (MS) media under sterile conditions for 4 weeks before carrying out molecular study according to the methods outlined by (Shukla and Sharma, 2017).

2.2 Genomic DNA Extraction

The Cetyltrimethylammonium bromide (CTAB) protocol optimized for the mucilage-rich Okra tissue was used to extract the genomic DNA from fresh young leaves collected from M1 generation plants (Doyle and Doyle, 1987: Bege et al., 2024). For DNA extraction, leaves obtained from seeds exposed to various distances and durations of radiation were initially snap-frozen at -70°C according to the methods of (Harrington et al., 2024). These frozen leaves were then ground into fine powder using mortar and pestle. 1 g of the powdered leaves was transferred separately into Eppendorf tubes after which 500 µL of extraction buffer containing 2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl at pH 8.0, 20 mM EDTA at pH 8.0, 1% polyvinylpyrrolidone, and 0.1% β-mercaptoethanol was added. The content was properly mixed using digital vortex mixer and then incubated at 65°C. After incubation, the samples were centrifuged at 15,000 rpm for 5 minutes at room temperature and the supernatant were transferred into clean tubes. An equal volume of chloroform: isoamyl alcohol in ratio 24:1 was added to the tubes, vortexed, and centrifuged again at 15,000 rpm for 5 minutes at room temperature. The resulting upper phase was carefully transferred to a fresh tube, and this step was repeated again to ensure purity.

For DNA precipitation, 350 µL of ice-cold 70% 2-propanol was added to the samples in each tube, gently vortexed, and incubated at -20°C for 15 minutes. This was thereafter centrifuged at 15,000 rpm for 10 minutes and the 2-propanol supernatant was carefully decanted to leave the DNA pellet in the tube. The pellet was then rinsed with 70% ethanol and centrifuged once again at 15,000 rpm for 5 minutes at room temperature. After draining the ethanol carefully, the pellet was allowed to dry by exposing it to air for approximately 30 minutes and each DNA pellet was resuspended in 20 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and incubated at 60°C for 5 minutes to facilitate dissolution.

2.3 DNA Quantification

The method outlined by García-Alegría et al. (2020) was adopted for assessing the concentrations and purities of the extracted DNA using NanoDrop™ spectrophotometer (JENWAS, Genova nano). The ratios of absorbance at 260 nm to 280 nm (A260/A280) and 260 nm to 230 nm (A260/A230) were considered for estimating the purity of DNA. As suggested by Gautam et al. 2025) values within the range of 1.8 – 2.0 for A260/A280 and 1.8 – 2.3 for A260/A230 were considered acceptable DNA purity for the study. According to them, lower values indicate presence of contaminants.

2.4 Polymerase Chain Reaction (PCR)

Two out of the five SCoT primers considered in this study showed polymorphism levels below the 50% threshold and were on this basis not considered for the study. To ensure reliable and effective assessment of genetic variability among treatments, the three primers that produced polymorphic bands exceeding the 50% threshold were selected for study (Table 1).

The Applied Biosystems Veriti™ Thermal Cycler was used for amplification of DNA fragment. Each PCR reaction contained 1 µL of genomic DNA template, 0.3 µL of forward primer, 0.3 µL of reverse primer, 0.3 µL of dNTPs, 0.075 µL of Taq DNA polymerase, 1.5 µL of 10X standard Taq buffer, and 11.525 µL of sterile distilled water as suggested by Mazlan et al. (2024). The PCR cycling conditions includes: an initial denaturation step at 94°C for 3 to 5 minutes; followed by 35 cycles comprising denaturation at 94°C for 30 seconds, annealing at 55°C for 60 seconds, and extension at 72°C for 30 to 60 seconds; a final extension at 72°C for 5 to 10 minutes; and a final hold at 4°C.

2% Agarose gel electrophoresis was used to confirm successful amplification of PCR products prior to fragment analysis. To ensure the accuracy and reliability of the results, each PCR run included both positive controls (using known DNA samples) and negative controls without DNA template.

2.5 Agarose Gel Electrophoresis

The PCR amplicons obtained for each marker were separated by electrophoresis on a 2% agarose gel at 100 volts as suggested by Rizk et al. (2024). The 2% agarose gel was prepared by dissolving 2 g of agarose in 100 mL of 1X Tris-acetate-EDTA (TAE) buffer follow by gentle microwaving. Once dissolved, the agarose solution was allowed to cool, then 10 µL of ethidium bromide was added for DNA visualization. This solution was poured into a gel casting tray fitted with a comb and left to solidify. The gel was submerged in 1X TAE buffer and samples were loaded alongside a 5.5 µL aliquot of a 100-base pair (bp) DNA ladder, and electrophoresis was run at 100 volts until adequate fragment migration was achieved. The amplified products were visualized using a high-performance UV transilluminator (VWR).

Table 1: Primers used for the studies and their sequence

Primer	Sequence	Reference
SCoT-1	5'-CAACAATGGCTACCACCA-3'	Satya et al. (2015)
SCoT-10	5'-CACCATGGCTACCACCAG-3'	Zhang et al. (2015)
SCoT-11	5'-ACCATGGCTACCACCGGC-3'	Mulpuri et al. (2013)

2.6 Data Analysis

To avoid ambiguity, only consistent bands within 100 bp to 1,000 bp were recorded. Also, bands clearly visible in at least one species were scored 1 (present) or 0 (absent) and recorded in a binary matrix. The similarity index proposed by Nei and Li (1979) was employed to determine similarity (Sab) between two cultivars, a and b, according to the formula:

$$Sab = {}^{2Nab}/_{(Na + Nb)}$$

where:
Nab = number of bands common to both samples a and b;

Na = number of bands in sample a;

Nb = number of bands in sample b.

A dendrogram (hierarchical cluster) was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and all analyses were performed using SPSS version 21 software.

3. Results

3.1 DNA Concentration and Purity

Figure 1 shows that the highest DNA concentration was recorded in the sample exposed to radiation from projector at a distance of 10 cm for 30 minutes, with a value of 2672 ng/µL which is higher than the values

recorded in control sample (2327 ng/µL). When the exposure distance was increased to 30 cm for 30 minutes, the DNA concentration decreased to 2070 ng/µL value that is lower than the control (2327 ng/µL). However, longer exposure at 10 cm for 1 hour reduced the DNA concentration of 1893 ng/µL while at 30 cm for 1 hour, the DNA concentration was slightly less than the control (2254 ng/µL) but higher than the other longer exposure groups.

All the treatments (including the control group) had absorbance values below 1.8 (Figure 2). The control sample has a purity ratio of 0.931 while sample exposed at 10 cm for 30 minutes had the highest absorbance of 1.069. Okra seeds exposed to radiation at 30 cm for 30 minutes had a lower purity ratio of 0.828 whereas longer exposure at 10 cm for 1-hour results in the lowest purity ratio of 0.771. Seeds placed at a distance of 30 cm from the projector for 1 hour had absorbance of 0.902 which is slightly higher than some other treatments.

Figure 3 shows the densitometric results of DNA content in okra samples exposed to projector radiation and the controls for the three SCoT markers studied. The control samples had low DNA percentages of 10.73% for SCoT-1, 9.02% for SCoT-10, and 11.72% for SCoT-11. However, exposure to radiation at a distance of 10 cm for 30 minutes notably increased the DNA content to 41.51%, 51.56%, and 56.81% for SCoT-1, SCoT-10, and SCoT-11 respectively. At 30 cm for 30 minutes, DNA content decreased compared to the closer exposure, with SCoT-1 remaining relatively high at 41.57%, but SCoT-10 and SCoT-11 dropping to 18.29% and 13.66%. Prolonged exposure of 1 hour at 10 cm distance from the projector resulted in a sharp decline in DNA content, especially for SCoT-1, which fell to 0.29%, while SCoT-10 and SCoT-11 decreased moderately to 14.79% and 12.17%. The lowest DNA content across all treatments was recorded for sample exposed to radiations at a distance of 30 cm for 1 hour, with values of 5.91%, 6.34%, and 5.64%.

3.2 Polymorphic Scoring of Bands

Table 2 and Figure 1A illustrate the presence or absence of seven DNA bands, ranging from 500 to 1000 bp, in okra samples exposed to projector radiation analyzed using the SCoT-1 marker. In the control group, bands 1, 3, 4, 6, and 7 were present, while bands 2 and 5 were absent. Exposure at 10 cm for 30 minutes resulted in the loss of band 1 but the appearance of bands 2 and 5; bands 3, 4, 6, and 7 remained present. At 30 cm for 30 minutes, bands 1, 2, 3, 4, 6, and 7 were present, with band 5 absent, similar to the control but with band 2 now present. Prolonged exposure at 10 cm for 1 hour caused the disappearance of all seven bands, showing complete loss of these DNA markers. At 30 cm for 1 hour, bands 2, 3, 4, 6, and 7 were present, while bands 1 and 5 were absent, resembling the pattern seen at 30 cm for 30 minutes.

Table 3 and Figure 1B show the presence or absence of six DNA bands, ranging from 300 to 700 bp, in okra samples exposed to projector radiation, analyzed using the SCoT-10 marker. In the control group, only band 4 was present, while all other bands were absent. Exposure at 10 cm for 30 minutes led to the appearance of bands 2, 3, 4, and 5, with bands 1 and 6 absent. At 30 cm for 30 minutes, bands 1, 3, and 6 were present, while bands 2, 4, and 5 were absent. For 10 cm exposure lasting 1 hour, bands 4 and 5 were present, with the rest absent. Finally, at 30 cm for 1 hour, only band 6 was present, and all others were absent.

Table 4 and Figure 1C illustrate the presence or absence of nine DNA bands in okra samples exposed to projector radiation within the range of 200 to 800 bp using the SCoT-11 marker. In the control group, bands 6, 7, 8, and 9 were present, while bands 1 through 5 were absent. Exposure at 10 cm for 30 minutes maintained the presence of bands 6 and 7 but bands 8 and 9 disappeared; bands 1 to 5 remained absent. At 30 cm for 30 minutes, bands 1, 2, 3, 4, 6, 7, 8, and 9 were present, with only band 5 absent, showing the greatest number of bands detected. For 10 cm exposure lasting 1 hour, bands 2, 3, 5, 6, and 7 were present, while bands 1, 4, 8, and 9 were absent. However, at 30 cm for 1 hour, only band 8 was present while other bands absent.

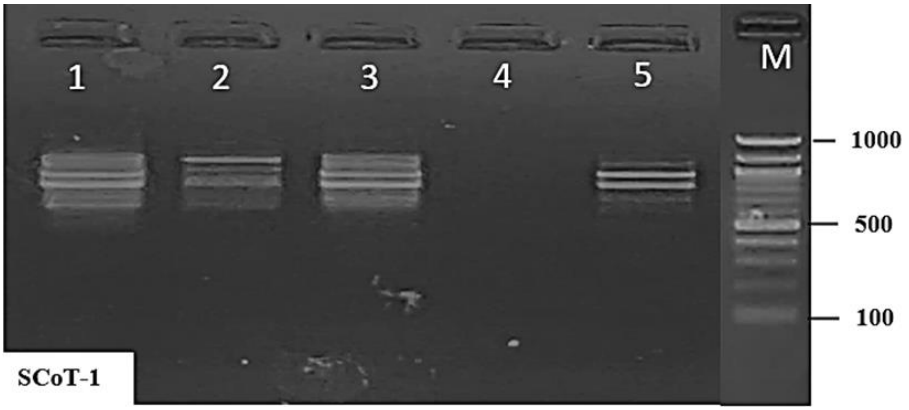
Table 5 shows the number of DNA bands detected in okra samples exposed to projector radiation at different distances and durations for the three SCoT primers: SCoT-1, SCoT-10, and SCoT-11. In the control group, a total of 10 bands were detected; 5 with SCoT-1, 1 with SCoT-10, and 4 with SCoT-11. Exposure at 10 cm for 30 minutes increased the total bands to 12, with 6 detected by SCoT-1, 4 by SCoT-10, and 2 by SCoT-11. At 30 cm for 30 minutes, the highest total number of bands (17) was observed, with 6 for SCoT-1, 3 for SCoT-10, and 8 for SCoT-11. Longer exposure at 10 cm for 1 hour resulted in fewer bands, totaling 7, with 0 for SCoT-1, 2 for SCoT-10, and 5 for SCoT-11. Exposure at 30 cm for 1 hour also yielded 7 bands in total, with 5 for SCoT-1, 1 for SCoT-10, and 1 for SCoT-11. Generally, 55 bands were detected across all the treatments: 22 for SCoT-1, 11 for SCoT-10, and 20 for SCoT-11.

Table 6 presents details of polymorphism, efficiency, and discriminatory values for three SCoT primers used on okra samples. SCoT-1 amplified a total of 22 bands, all of which were polymorphic with no unique or monomorphic bands, resulting in 100% polymorphism. Its efficiency was 41.51, and the discriminatory value was 44.90%. SCoT-10 amplified 11 bands, including 2 unique bands and 9 polymorphic bands, with no monomorphic bands. The percentage of polymorphic bands was 81.82%, efficiency was 20.75, and the discriminatory value was 18.37%. SCoT-11 amplified 20 bands, including 2 unique bands and 18 polymorphic bands, with no monomorphic bands. It showed 90% polymorphism, an efficiency of 37.74, and a discriminatory value of 36.73%.

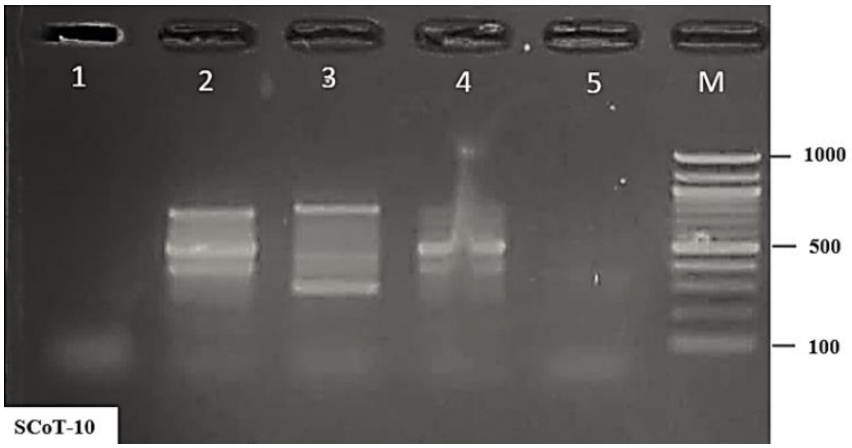
3.3 Genetic Divergence Pattern of the treated okra samples

Table 7 presents the similarity coefficients among okra samples exposed to projector radiation under the different treatments considered in this study. The control sample showed similarity values of 0.64 with the 10 cm for 30 minutes treatment, 0.67 with the 30 cm for 30 minutes treatment, 0.35 with the 10 cm for 1 hour treatment, and 0.59 with the 30 cm for 1 hour treatment. Among the treatments, the similarity between 10 cm for 30 minutes and 30 cm for 30 minutes was 0.55, while between 10 cm for 30 minutes and 10 cm for 1 hour it was 0.42. The similarity between 10 cm for 30 minutes and 30 cm for 1 hour was 0.52. Between 30 cm for 30 minutes and 10 cm for 1 hour, the similarity was 0.33, and between 30 cm for 30 minutes and 30 cm for 1 hour, it was 0.58. The lowest similarity was observed between 10 cm for 1 hour and 30 cm for 1 hour, with a value of 0.00, indicating no genetic similarity in their effects.

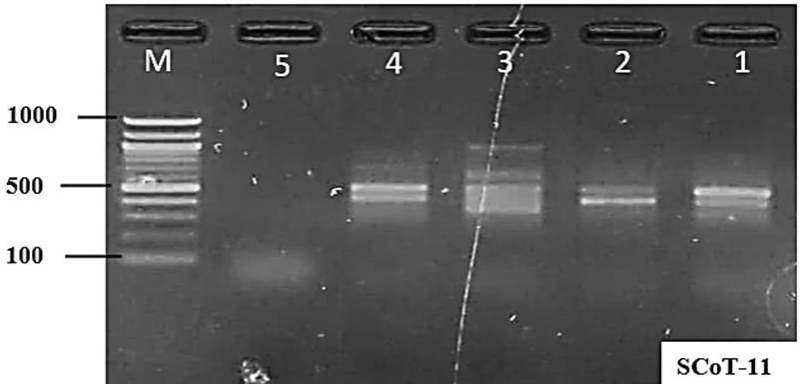
Figure 4 presents a dendrogram obtained from the DNA profiles of Okra samples exposed to varying distances and durations of projector radiation and the control. The treatments clustered into two main groups, with the sample exposed to radiation at 10 cm for 1 hr solely occupying the first cluster. The second cluster comprised of control (sample not exposed to radiation), samples exposed to radiation at 30 cm for 1 hour, 10 cm for 30 minutes, 30 cm for 30 mins. More specifically, the second cluster is divided into three sub-clusters: the first contains untreated control samples and samples exposed at 30 cm for 30 mins and the untreated control, while the second comprised of sample exposed at 10 cm for 30 minutes. The last sub-cluster comprised of okra exposed to projector radiation at a distance of 10 cm for 1 hour.



A



B



C

Figure 1 A-C: Electrophoretic DNA Band Patterns in Okra (*Abelmoschus esculentus* L.) Subjected to Different Distances and Exposure Durations of Projector Radiation Using Three SCoT Markers

Key:

1: Control

2: 10cm for 30min

3: 30cm for 30min

4: 10cm for 1hr

5: 30cm for 1hr

M: Marker

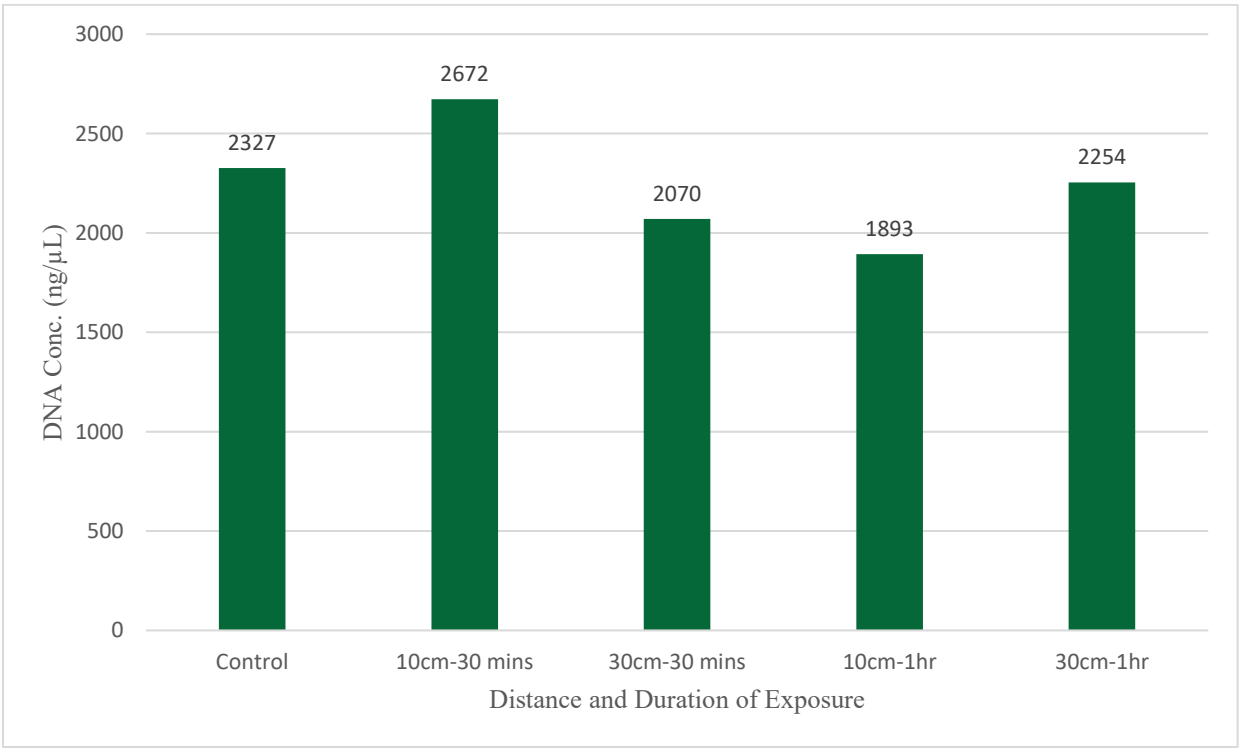


Figure 2: DNA Concentration in Okra (*Abelmoschus esculentus* L.) Exposed to Projector Radiation at Various Distances and Exposure Durations

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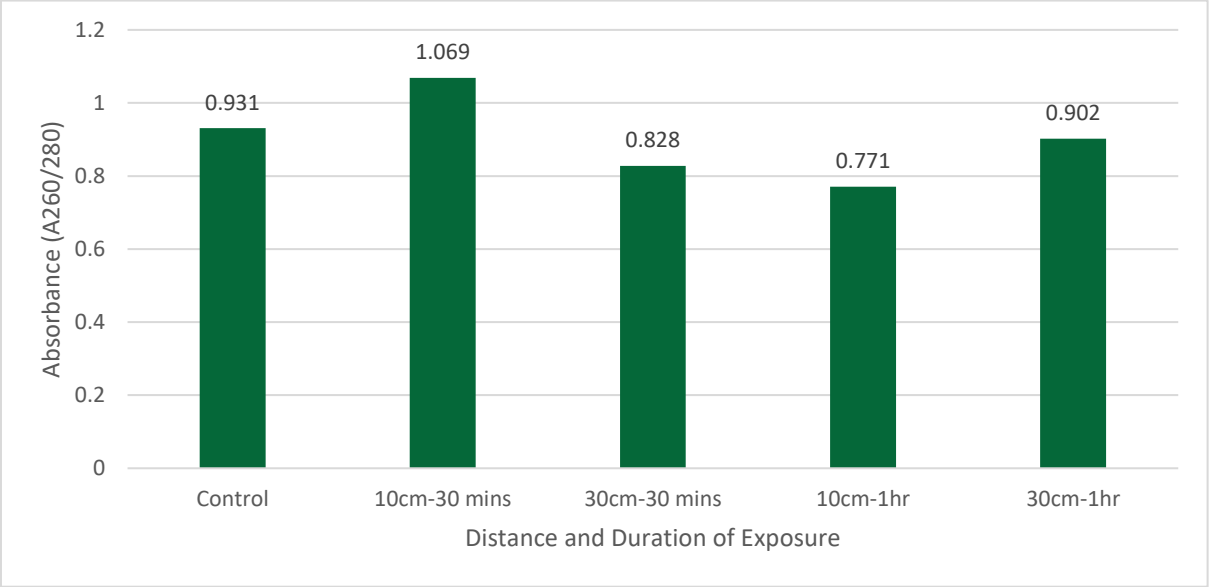
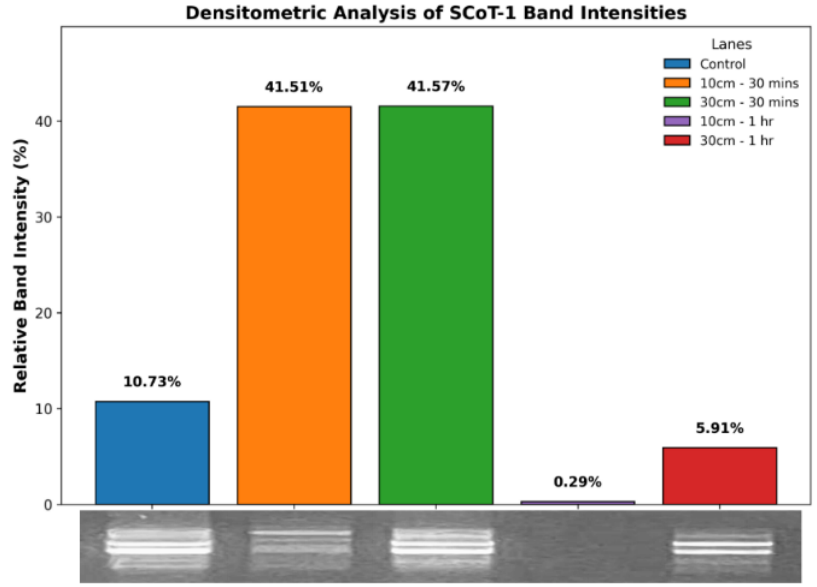


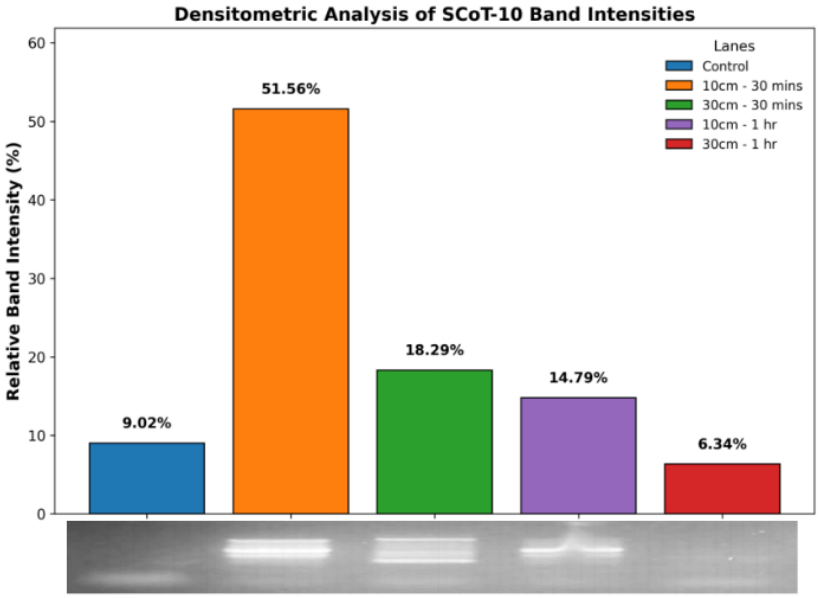
Figure 3: Purity Levels of DNA Extracted from Okra (*Abelmoschus esculentus* L.) Subjected to Projector Radiation at Varying Distances and Exposure Durations

Note:

1.8 and above: Acceptable DNA purity

Below 1.8: Presence of protein impurities





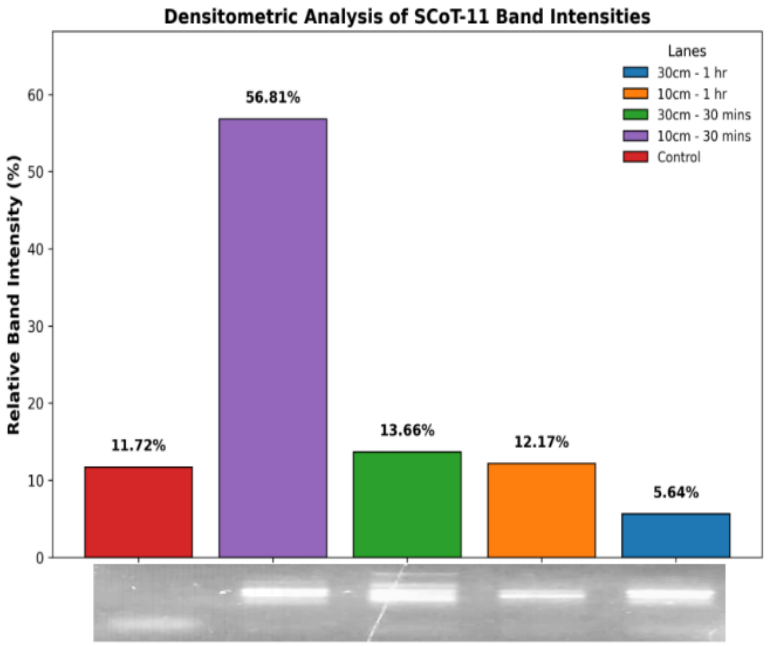


Figure 4: Densitometric Analysis of DNA Band Intensities from the SCoT Markers

Table 2: Bands Present in Okra (*Abelmoschus esculentus* L.) Exposed to Projector Radiations using SCoT-1 marker							
Treatments	**Band 1**	**Band 2**	**Band 3**	**Band 4**	**Band 5**	**Band 6**	**Band 7**
Control	1	0	1	1	0	1	1
10cm-30mins	0	1	1	1	1	1	1
30cm-30mins	1	1	1	1	0	1	1
10cm-1hr	0	0	0	0	0	0	0
30cm-1hr	0	1	1	1	0	1	1

Table 3: Bands Present in Okra (*Abelmoschus esculentus* L.) Exposed to Projector Radiations using SCoT-10 marker						
Treatments	**Band 1**	**Band 2**	**Band 3**	**Band 4**	**Band 5**	**Band 6**
Control	0	0	0	1	0	0
10cm-30mins	0	1	1	1	1	0
30cm-30mins	1	0	1	0	0	1
10cm-1hr	0	0	0	1	1	0
30cm-1hr	0	0	0	0	0	1

Table 4: Bands Present in Okra (Abelmoschus esculentus L.) Exposed to Projector Radiations using SCoT-11 marker									
Treatments	**Band 1**	**Band 2**	**Band 3**	**Band 4**	**Band 5**	**Band 6**	**Band 7**	**Band 8**	**Band 9**
Control	0	0	0	0	0	1	1	1	1
10cm-30mins	0	0	0	0	0	1	1	0	0
30cm-30mins	1	1	1	1	0	1	1	1	1
10cm-1hr	0	1	1	0	1	1	1	0	0
30cm-1hr	0	0	0	0	0	0	0	1	0

0 = absence of band 1 = presence of band

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Table 5: Number of Bands Detected in Okra Exposed to Projector Radiation at Different Distances and Durations Using the SCoT Primers

Treatments	SCoT-1	SCoT-10	SCoT-11	Total No of Bands
Control	5	1	4	10
10cm for 30mins	6	4	2	12
30cm for 30mins	6	3	8	17
10cm for 1hr	0	2	5	7
30cm for1hr	5	1	1	7
Total	22	11	20	53

Table 6: Polymorphism Details, Efficiency and Discriminatory Values for the 3 SCoT Primers

Treatments	Total no of bands amplified	No of unique bands	No of Polymorphic Bands	No of Monomorphic Bands	Percentage of Polymorphic Bands (%)	Efficiency	Discriminatory value (%)
SCoT-1	22	0	22	0	100	41.51	44.90
SCoT-10	11	2	9	0	81.82	20.75	18.37
SCoT-11	20	2	18	0	90	37.74	36.73
Total	53	4 (7.55%)	49 (92.45 %)	0		100%	100%

Table 7: Similarity Indices for Okra Exposed to Projector Radiation at Different Distances **and Durations Using the SCoT Primers**

Treatment	Control	10cm for 30mins	30cm for 30mins	10cm for 1hr	30cm for 1hr
Control	-				
10cm for 30mins	0.64	-			
30cm for 30mins	0.67	0.55	-		
10cm for 1hr	0.35	0.42	0.33	-	
30cm for1hr	0.59	0.52	0.58	0.00	-

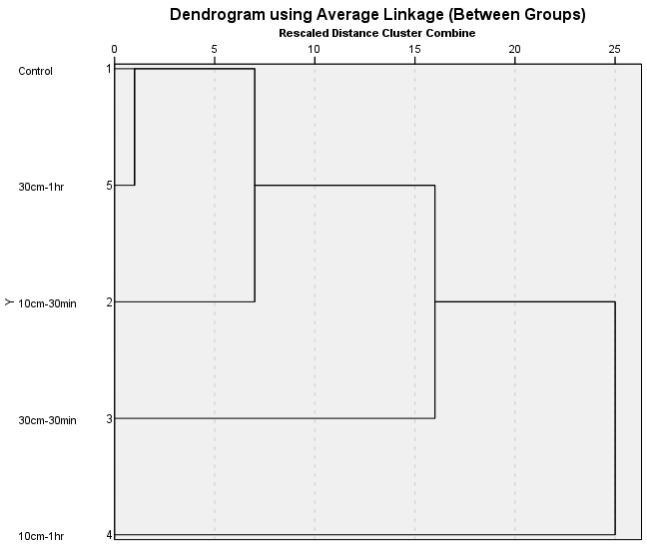

Figure 5: Genetic Similarity of Okra Exposed to Projector Radiation at Varying Distances and Durations Using SCoT Indicators

4. Discussion

Genetic diversity within species forms the basis of effective breeding programs by providing raw material for superior trait selection and improved cultivar development (Altaf et al., 2025). This makes radiation-induced variation particularly valuable for okra improvement.

4.1 DNA Quality and Quantity

DNA concentration and purity were strongly influenced by the doses of radiation as well as exposure duration. Moderate exposure at 10 cm for 30 minutes significantly increased DNA concentration and slightly improved purity compared to controls which indicates stimulatory effect of projector radiation on DNA integrity. As reported similar dose-dependent effect on DNA integrity of *Gladiolus grandiflorus* irradiated with red laser light (Hassan et al., 2024). However, prolonged exposure (particularly at 10 cm for 1 hour) resulted in a marked decrease in DNA concentration and purity which suggests radiation-induced DNA degradation. This

observation agrees with findings who reported significantly high DNA loss in ovaries irradiated with high-energy electrons at 10 Gy (Miler et al., 2023). The generally suboptimal purity ratios across all treatments including unirradiated samples support the known challenges of extracting high-quality DNA from okra which could be attributed to high mucilage and polyphenolic contents of the crop according to (Johar et al., 2024).

4.2 Polymorphism induction

In this study, SCoT marker analysis revealed that projector radiation induces high levels of DNA polymorphism with percentages ranging from 81.82% to 100% depending on primer and treatment. This range is higher that the percentages reported that SCoT markers showed 85.05% polymorphism, and SRAP markers showed 70.59% polymorphism for chickpea irradiated with gamma ray by (Harb et al., 2025). The observed variation in band numbers and patterns demonstrates that intermediate projector radiation treatments (e.g., 10 cm for 30 minutes and 30 cm for 30 minutes) effectively induce genetic changes, possibly through activation of silent genomic regions or mutation generation. In contrast to this, longer exposure (10 cm for 1 hour) retained high polymorphism but showed band loss which indicates selective genomic region activation or suppression. It should be noted that treatments such as 30 cm for 1 hour and controls lacked detectable banding which suggests either insufficient radiation dose to induce changes or degradation of DNA fragments. As reported up to 11% DNA loss in ovaries of Chrysanthemum exposed to 10 Gy of high-energy radiation (Miler et al., 2023). The fact that the highest number of DNA bands (17) was observed in samples exposed to projector radiation at a distance of 30 cm for 30 minutes further affirms that moderate exposure can also induce DNA polymorphism and genetic diversity in okra. In this study, prolonged exposure of samples to projector radiation resulted in drastic reduction in band count, which can be attributed to the earlier reported radiation-induced DNA damage. This damage collectively leading to significant decrease in the detectable intact DNA fragments during molecular analyses according to (Liu et al., 2009).

The SCoT-1 primer produced the highest number of DNA bands and revealed clear polymorphisms which indicates its suitability for detecting genetic diversity in okra. This supports the earlier report of Bidyananda et al. (2024) that primers generating more polymorphic bands tend to provide more reliable and informative assessments of genetic diversity in plants. Although the SCoT-10 and SCoT-11 primers were still useful, they produced fewer bands and less consistent polymorphic patterns, making them comparatively less informative for detailed evaluation of genetic diversity of okra.

4.3 Genetic Divergence Pattern

Genetic similarity result and dendrogram clustering further supported dose- and exposure-dependent genetic divergence pattern. So, samples exposed to intense radiation (10 cm for 1 hour) clustered distinctly from control and milder treatments which suggests maximal genetic differentiation. The close similarity between unirradiated controls and samples exposed at a distance of 30 cm for 1-hour indicates negligible mutagenesis under those conditions. In addition to this, the unique clustering of samples exposed to projector radiation at a distance of 30 cm for 30 minutes samples, which earlier exhibited 100% polymorphism and unique banding, highlights that moderate-duration exposures at greater distances can induce distinct genetic variation. These results correspond well with prior findings where gamma radiation generated dose-dependent genomic alterations and polymorphism profiles (Gautam et al., 2025). Similarly, as reported that genetic variation induced among regenerated Chrysanthemum species correlated strongly with the type and dose of ionizing radiation used (Miler et al., 2023).

Generally, findings from this study confirms projector radiation as an effective physical mutagen capable of inducing substantial genetic variability in okra. The polymorphic banding patterns and genetic diversity generated by intermediate doses of projector radiation establish a molecular basis to support previously reports of the morphological changes and cytogenetic alterations in Okra and Onion respectively (Alege et al., 2022a; Alege et al., 2022b). These findings support the strategic application of projector radiation to increase genetic diversity during okra breeding programs, and other self-pollinating crops where limited natural variations have been reported.

5. Conclusion

Projector radiation have been found to significantly influences DNA concentration, purity, and genetic diversity in okra with samples positioned at a distance of 10 cm for 30 minutes enhancing genetic polymorphism while minimizing DNA degradation. These findings identify projector radiation as a potent, affordable mutagen for inducing genetic variability for okra improvement. It is advisable to avoid prolonged or close exposures that could cause DNA damage. Integrating projector radiation into mutation breeding protocols can help to accelerate the development of superior okra varieties. Future studies should consider evaluating the genetic variability induced by projector radiation using other molecular markers as well as assessing the heritability of these variabilities across generations to validate its suitability for use as physical mutagen.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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