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Molecular Diversity Analysis of *in vitro* and Irradiated Tomato (*Lycopersicon esculentum* Mill) Grew Under Salt Stress Expressed by SCoT and ISSR Markers

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ABSTRACT

Tomato buds of cv. Idkawy were cultured *in vitro* on solid MS medium with 0.2 mg^{-1} BAP. The plantlets that were produced were exposed to different doses of gamma radiation, ranging from 100 to 200 Gy. Afterward, single pieces of nodes were cut and moved to a fresh MS medium with 0.2 mg-l of BAP. The gamma radiation caused a mortality rate of 18.75% to 52.5% among the explants. The surviving plantlets were then cut into single node pieces and transferred to an MS medium containing 0.2 mg-l of BAP, with added NaCl concentrations of either 50 or 100 mM. There was increased mortality of the vegetative buds on the explants with increased salt concentrations. It was shown that the all gamma radiation doses caused reduced the percentage of survival at saline levels. The genetic diversity was assessment by using ten primers for each SCoT and ISSR markers to six irradiated treatments grew under salt stress (100 Gy x 50 mM, 150 Gy x 50 mM, 200 Gy x 50 mM, 100 Gy x 100 mM, 150 Gy x 100 mM, 200 Gy x 100 mM). It was showed that the polymorphism percentage mean of SCoT marker (29.56%) is higher than the ISSR marker (26.78%). The average of *PIC* values for both markers SCoT and ISSR were 0.197 and 0.288 (PIC <0.5), as well as, *MI* values were 0.077 and 0.081, respectively. In contrast, when considering the number of alleles (Ne), Nei's genetic diversity (H), and Shannon's information index (I) parameters, it was observed that the greatest genetic variation was caused by the combined treatment of 200 Gy x 50 mM NaCl using the SCoT marker. On the other hand, with the ISSR marker, the highest induced genetic variation was seen with the combined treatment of 150 Gy x 50 mM NaCl. The obtained results demonstrate that SCoT marker was more accurate and efficient than ISSR marker for distinguishing and genetic variation analysis of irradiated tomato plantlets grew under salt stress. The relationships within treatments were estimated through cluster analysis (UPGMA) based on SCoT and ISSR analysis.

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Introduction

Tomato (*Solanum lycopersicum* Mill) is an important crop worldwide due to its nutritional value and economic significance. In recent years, the study of plant response to environmental stressors has gained significant attention due to its implications for agriculture and food security FAO (2019). Gamma radiation and salinity are two such stressors that can affect plant growth and development. It is essential to comprehend the collective impacts of these stresses on tomato plants, given that tomatoes are among the most extensively grown and consumed fruits worldwide according to USDA (2020).

In vitro culture techniques, such as tissue culture and micropropagation, are commonly employed for the mass production of disease-free and genetically uniform plantlets. These techniques offer a controlled environment where the effects of salt stress and gamma radiation can be studied in a controlled and reproducible manner (Forster and Shu, 2012; Roychowdhury and Tah, 2013).

However, tomato plants are highly susceptible to abiotic stresses such as salt stress, which can significantly reduce their growth and yield (Lobell and Gourdji, 2012; Hasanuzzaman et al. 2013). Gamma radiation, a form of ionizing radiation, can elicit various physiological and genetic changes in plants.

It can induce DNA damage, alter gene expression patterns, affect photosynthetic efficiency, and modulate antioxidant defense mechanisms (Kodym and Afza 2003; Ezzat et al. 2019; Tiwari and Singh, 2019). On the other hand, salinity stress arises when soil or water contains high concentrations of salt, mainly sodium chloride. Salinity stress negatively affects plant growth and development by disrupting osmotic balance, ion homeostasis, nutritional imbalances, and oxidative stress in plants (Kumar and Sharma, 2017; Yadav and Arora, 2019; Ashraf and Ozturk, 2021). Exploring the simultaneous impact of salt stress and gamma radiation on in vitro-grown tomato plants is essential for devising strategies to improve their resilience to stress and broaden their genetic variability. This research can also uncover any potential cooperative or conflicting interactions between salt stress and gamma radiation. Additionally, examining the combined influence of these stressors can facilitate the creation of tomato varieties that are better equipped to withstand stress while also possessing a wider genetic diversity (Mittler, 2006; IAEA, 2012). While previous studies have looked into the effects of salt stress or gamma radiation on in vitro-grown tomato plants independently, there is a lack of research on how these two factors work together in affecting tomato plants Jazayeri et al. (2016). Genetic markers play a crucial role in modern plant genetics and breeding, enabling researchers to study genetic diversity, population structure, and trait inheritance. These markers provide valuable information about the genome of organisms, allowing for the identification of specific genes or regions associated with desirable traits Collard et al. (2005). Among the various types of genetic markers, Start Codon Targeted (SCoT) and Inter Simple Sequence Repeats (ISSR) markers have attracted considerable interest because of their flexibility and efficiency in genetic analysis, as highlighted by (Collard and Mackill, 2009).

The use of SCoT and ISSR markers has several advantages over other marker systems. They are dominant markers, meaning they can be easily scored without the need for complex genotyping techniques (Gupta and Varshney, 2000). Additionally, they have high levels of polymorphism, making them suitable for studying diverse plant populations. Moreover, SCoT and ISSR markers are PCR-based, allowing for efficient and rapid analysis of large numbers of samples Madhava Rao et al. (2006). Therefore, the present study aims to investigate the effect of salt stress and gamma radiation on tomato plants grown in vitro. This research will assess the impact of different levels of salt stress in combination with different doses of gamma radiation on growth parameters, morphological characteristics, biochemical changes, and gene expression patterns in in vitro-grown tomato plants. The findings of this study will contribute to our understanding of the combined stress response of tomato plants and can inform strategies for enhancing their stress tolerance and genetic diversity.

Materials and Methods

Seed Material

The tomato seeds, specifically the Idkawy cultivar, were procured from the Vegetable Research Institute, Agricultural Research Centre, Ministry of Agriculture in Egypt.

Application of Gamma Rays Irradiation

In order to start the process of irradiation, a ^{137}Cs source was utilized with a dose rate of 1 Gy every 2 minutes and 30 seconds. This procedure took place at the National Centre for Radiation Research and Technology, which is part of the Egyptian Atomic Energy Authority, located in Cairo, Egypt. To conduct the experiment, tomato seeds were soaked in water and exposed to different doses of gamma radiation, specifically 100, 150, and 200 Gy. A total of 80 seeds were exposed to gamma rays at each dose. Following irradiation, the treated seeds underwent sterilization by being immersed in a 30% Clorox solution for ten minutes, followed by three rinses in sterile distilled water. After the sterilization process, these treated seeds were then cultured on a solid MS medium, also known as Murashige and Skoog medium (1962), which does not contain any additional hormones. Approximately 6-8 weeks later, micropropagation began once the plantlets reached a height of around 10-12 cm. To maintain the culture, the plantlets were divided into individual nodes and transferred to MS medium supplemented with 0.2 mg-l BAP (benzylaminopurine). Before autoclaving, the pH level of the culture medium was adjusted to 5.7. Subsequently, the buds were placed in a growth chamber with a temperature of 25°C ± 2, and a photoperiod of 16 hours.

Salt Tolerance Selection after Irradiation

In order to assess the plants' tolerance to salt following irradiation, the individual nodes were transferred to fresh MS solid medium enriched with 50 and 100 mM NaCl, along with 0.2 mg^{-l} BAP. The survival rate of these single node cuttings was recorded after a period of 6-8 weeks.

Genomic DNA Extraction

In order to acquire complete genomic DNA from plantlets that have been exposed to radiation, we employed the extraction procedure outlined by Anderson et al. (1992), making a few adjustments to improve the quality of the DNA. Our approach involved carrying out two successive extractions utilizing a solution consisting of phenol and chloroform in a 1:1 ratio. Following this, we performed an additional cleaning step using 97% alcohol, which was stored at -20°C for a duration of one hour. Afterwards, we conducted a pre-cooled ethanol wash with a concentration of 70%. To assess the DNA's quantity and quality, we utilized gel electrophoresis.

SCoT – PCR Amplification

We have chosen ten SCoT primers for PCR amplification, based on the method described by Collard and Mackill (2009) (Table 1). The amplification reactions were conducted in a total volume of 25 µl. Each reaction consisted of 250 µM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM of $MgCl_2$, 1 unit of Taq polymerase, and 50-100 ng of template DNA. A drop of mineral oil was added on top of all reaction volumes. The thermocycling program began with an initial cycle at 94°C for 3 minutes, followed by 35 cycles at 94°C for 50 seconds, 1 minute at 50°C, and 2 minutes at 72°C. Finally, there was a 7-minute extension step at 72°C. To observe the amplified product from PCR, we performed electrophoresis on a 1.0% agarose gel and visualized the amplified fragments by staining them with ethidium bromide.

Table 1. Primers code and nucleotide sequences of the ten used SCoT and ISSR primers.

SCoT				ISSR		
No.	Marker	Sequences (5′-3′)	% GC	Marker	Sequences (5′-3′)	Repeat motif
1	SCoT- 1	5'-CAACA**ATG**GCTACCACCA-3'	50	ISSR1	5'-AGAGAGAGAGAGAGAGYC-3'	(AG)8 YC
2	SCoT- 2	5'-CAACA**ATG**GCTACCACCC-3'	56	ISSR2	5'-AGAGAGAGAGAGAGAGYG-3'	(AG)8 YG
3	SCoT- 3	5'-CAACA**ATG**GCTACCACCG-3'	56	ISSR4	5'-ACACACACACACACACYG-3'	(AC)8 YG
4	SCoT- 4	5'-CAACA**ATG**GCTACCACCT-3'	50	ISSR5	5'-GTGTGTGTGTGTGTGTYG-3'	(GT)8 YG
5	SCoT- 5	5'-CAACA**ATG**GCTACCACGA-3'	50	ISSR7	5'-ACGATAGATAGATAGATA-3'	GAC(GATA)4
6	SCoT-12	5'-ACGAC**ATG**GCGACCAACG-3'	61	ISSR11	5'-ACACACACACACACACYA-3'	(AC)8 YA
7	SCoT-13	5'-ACGAC**ATG**GCGACCATCG-3'	61	ISSR12	5'-ACACACACACACACACYC-3'	(AC)8 YC
8	SCoT-16	5'-ACC**ATG**GCTACCACCGAC-3'	56	ISSR13	5'-AGAGAGAGAGAGAGAGYT-3'	(AG)8 YT
9	SCoT-20	5'-ACC**ATG**GCTACCACCGCG-3'	67	ISSR14	5'-CTCCTCCTCCTCCTCTT-3'	(CTC)5 TT
10	SCoT-33	5'-CC**ATG**GCTACCACCGCAG-3'	67	ISSR24	CAC CAC CAC GC	(CAC)3 GC

ISSR – PCR Amplification

The amplification process of ISSR – PCR was carried out utilizing a collection of ten distinct ISSR primers. These primers, designed specifically to target di-, tri-, or tetra-nucleotide SSR repeats, consist of either 11 or 18 nucleotides. Additionally, each primer includes a selective anchor sequence of 2 nucleotides ′3. The chosen ISSR primers are as follows: (AG)8 YC, (AG)8 YT, (AG)8 YG, (AC)8 YG, (AC)8 YC, (AC)8 YA, (GT)8 YG, (CTC)5 TT, (CAC)3 GC, and GAC(GATA)4 (refer to Table 1). For the amplification reactions, a total volume of 25 µl was used, consisting of the following components: 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl2, 200 µM of each dNTP, 1 µM of primer, 30 ng of genomic DNA, and 1.5 U of Taq DNA polymerase. To initiate the process, the reaction mixture was overlaid with two drops of mineral oil and subjected to an initial denaturation at 95°C for 3 minutes. Following this, the amplification process involved 45 cycles, each consisting of 30 seconds at 94°C, 30 seconds at 45°C, and 60 seconds at 72°C. Finally, the reaction was incubated at 72°C for 7 minutes. The resulting amplification products were separated using gel electrophoresis on a precast 0.8% agarose gel. After staining with ethidium bromide, the products were observed under UV illumination and captured through photography.

Data Analysis

Fragment sizes for both SCoT and ISSR markers were determined using PyElph 1.4 software, developed by (Pavel and Vasile, 2012). The amplified products were classified as present (1) or absent (0), resulting in a binary matrix. To assess the markers' ability to differentiate between genotypes, two metrics were calculated: the polymorphism information content (PIC) and the marker index (MI). PIC was computed according to Anderson et al. (1992) formula, where PIC = 1 - Σp¡2, with pi representing the frequency of the *i*th allele of the locus in six gamma radiation treatments. MI was calculated using the approach outlined by Varshney et al. (2007), which is the product of PIC and the effective multiplex ratio. For the analysis of genetic variation, additional parameters such as the effective number of alleles (Ne), Nei's gene diversity (H), and Shannon's information index (I) were determined using PopGen 1.3.1 software developed by Yeh et al. (1990). Jaccard's similarity coefficient was used to generate a similarity matrix, and the UPGMA algorithm was applied for hierarchical cluster analysis and to construct a dendrogram. These analyses were conducted utilizing MVSP, Ver 3.1 software created by (Kovach, 1998).

Results and Discussion

The combined impact of gamma irradiation doses (100, 150 and 200 Gy), and NaCl concentrations (50 and 100 mM) on the percent of microcutting survival, plantlets growing numbers and mean of shoot length were summarized in Table 2. The untreated plantlets grew well on NaCl free medium (92.5%), while the percent survival of single node buds, plantlets growing numbers and mean of shoot length (Figure 1) grown on MS medium containing distinctive concentrations of NaCl (50 & 100 mM) decreased to 76.25 & 66.25%; 61 & 53 plantlet and 4.2 & 3.9 cm respectively, as illustrated in Table 2. Salinity stress significantly affects tomato plants grown in vitro. High salt concentrations in the growth medium can reduce seed germination rates, inhibit root and shoot growth, induce leaf wilting, and negatively impact overall plant health. It disrupts ion balance, leading to a buildup of sodium ions within plant tissues, which can cause a nutrient imbalance and hinder essential physiological processes (Roșca et al. 2023; Villegas et al. 2023).

Table 2. The combined effect of gamma irradiation and salinity on tomato cv. Idkawy survival of micropropagated buds, plantlets growing No. and mean of shoot length.

Parameters	Treatments											
	Control	Salinity		Gamma radiation doses			Combined effect between gamma radiation doses (Gy) and salinity (mM)					
	0	A	B	C	D	E	F	G	H	I	J	K
% Bud survival	92.5	76.25	66.25	73.75	50	40	45	36.25	35	21.25	16.25	10
Plantlets growing No.	74	61	53	59	40	32	36	29	28	17	13	8
Mean of shoot length (cm)	5.3	4.2	3.9	2.3	2	1.54	3.9	3.5	3.2	3	2.8	1.88

A: 50 Mm; B 100 Mm; C: 100 Gy; D: 150 Gy; E: 200 Gy; F: 100 Gy × 50 Mm; G: 150 Gy × 50 Mm; H: 200 Gy × 50 Mm; I: 100 Gy × 100 Mm; J: 150 Gy × 100 Mm; K: 200 Gy × 100 mM

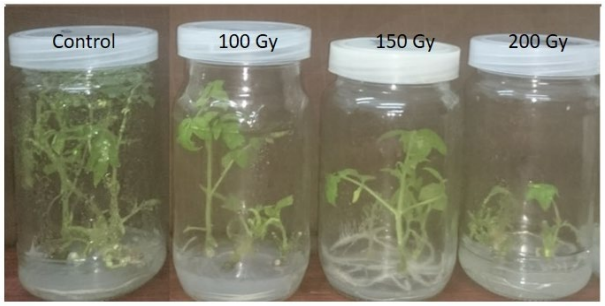

Figure 2. Effect of gamma irradiation doses (100, 150& 200 Gy) on survival and growth to tomato plantlets

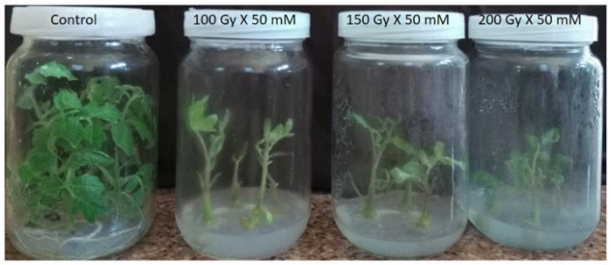

Figure 3. The combined effect of gamma irradiation doses (100, 150& 200 Gy) on survival and growth to tomato plantlets grew under 50 mM NaCl.

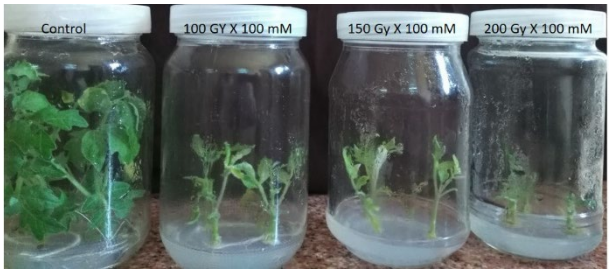

Figure 4. The combined effect of gamma irradiation doses (100, 150& 200 Gy) on survival and growth to tomato plantlets grew under 100 mM NaCl.

Salinity stress also triggers oxidative stress within tomato plants. Reactive oxygen species (ROS) are generated, resulting in cellular damage. Photosynthetic efficiency is reduced, leading to a decline in the production of chlorophyll, carbohydrates, and other primary metabolites. Additionally, salt-induced osmotic stress hampers water uptake, resulting in water deficit and subsequent plant dehydration Fahmy et al. (2020).

As well as, gamma irradiation doses of 100, 150 and 200 Gy caused a decrease in percent survival of micropropagated buds, plantlets growing numbers and mean of shoot length (Figure 2), to 73.75, 50 & 40%; 59, 40 & 32 plantlet; 2.3, 2 & 1.54 cm respectively, as shown in Table 2. Studies investigating the impact of gamma radiation on tomato plants grown in vitro have revealed both positive and negative effects. Low-dose gamma radiation has been reported to stimulate seed germination, enhance growth, improve plant morphology, increase fruit yield, and enhance nutrient uptake Pronabananda et al. (2021). These positive effects may be attributed to the hormetic response, a phenomenon where low doses of stressors result in beneficial effects on organisms. However, higher doses of gamma radiation can be detrimental, causing reduced growth, leaf chlorosis, decreased photosynthetic activity, and abnormal plant development. Moreover, gamma radiation can induce DNA damage and genomic instability. It can lead to mutations, chromosomal aberrations, and alterations in genetic material within tomato plants. These genetic changes can have long-term implications on plant fitness, adaptability, and overall growth performance El-Fiki et al. (2021). For instance, in a study by Moustafa and Abu El-Nasr (2015), tomato plants were exposed to different doses of gamma radiation (0, 25, 50, 75, and 100 Gy). The results indicated that low doses of gamma radiation (25 and 50 Gy) stimulated seed germination, seedling growth, and chlorophyll content, while higher doses (75 and 100 Gy) had inhibitory effects on these parameters. The study also revealed an increase in genetic variability and morphological changes in the irradiated tomato plants.

The impact of gamma irradiation doses (100, 150 & 200 Gy) on survival of buds, plantlets growing numbers and mean of shoot length growing onto MS medium containing 50 mM NaCl (Figure 3) were decreased to 47.5, 56.25 & 57.5%; 38, 45 & 46 plantlet and 1.4, 1.8 & 2.1 cm respectively. Whereas the use of the same gamma irradiation doses on the concentration 100 mM NaCl (Figure 4), it had a severe effect on survival of buds, plantlets growing numbers and mean of shoot length where decreased to 71.25, 76.25 & 82.5%; 57, 61 & 66 plantlet and 2.3, 2.5 & 3.42 cm respectively, Table 2. The combined effects of gamma radiation and salinity on in vitro tomato growth have been less extensively studied. However, it can be anticipated that simultaneous exposure to these stressors may elicit synergistic or antagonistic responses, depending on the dose and duration of exposure. It is plausible that the detrimental effects observed individually may exacerbate when plants are subjected to both stressors concurrently (Taha and Shoaib, 2021; Ulukapi, 2021).

Molecular Markers

The genetic variation in irradiated tomato plants, exposed to salt stress, was observed through the analysis of amplified DNA products. The genomic DNA from six treated tomato plants (cv. Idkawy) exposed to different doses of gamma rays (100, 150, and 200 Gy) and grown under varying NaCl concentrations (50 and 100 mM) was used as a basis for studying genetic diversity using SCoT and ISSR markers. The results of the SCoT and ISSR markers analysis, which examined the combined effects of gamma rays irradiation and salt stress, have been summarized in Table 3.

SCoT Marker Analysis

Based on the aforementioned findings and as indicated in (Table 3), Ten SCoT primers produced a total of 115 amplicons as shown in Figure 5. Each primer yielded a varying number of bands ranging from 6 to 15, with an average of 11.5 fragments per primer. Among the primers, SCoT-12 yielded the highest number of bands (15), while SCoT-4 resulted in the lowest number (6). The level of polymorphism ranged from 0% (ScoT-4 and SCoT-5) to 53.8% (SCoT-20), the polymorphism rate was found to be 29.56% on average in the study. The sizes of the amplified fragments ranged from 153 bp (SCoT-12) to 1594 bp (SCoT-16).

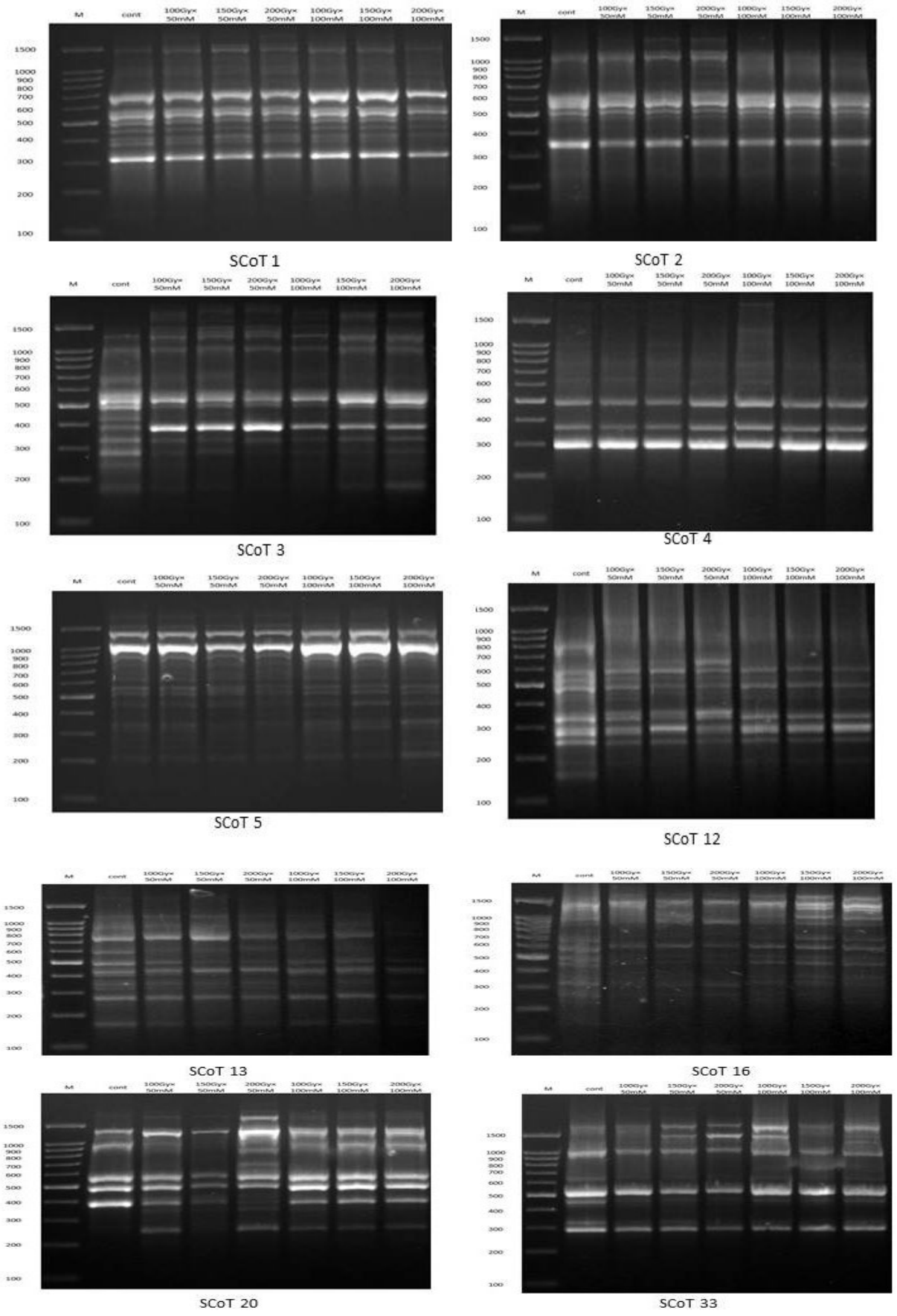

Figure 5. Representative profile of six combined effect between gamma radiation doses and salinity in tomato cv. Idkawy revealed SCoT marker.

Table 3. Amplification results generated by SCoT and ISSR primers in irradiated tomato plantlets

Marker													
SCoT							ISSR						
	TAB	PBN	%PB	BZ/bp	MI	PIC		TAB	PBN	%PB	BZ/bp	MI	PIC
SCoT- 1	12	1	8.3	295-1448	0	0	ISSR-1	12	2	16.6	215-390	0.03	0.18
SCoT- 2	10	3	30	227-1138	0.096	0.32	ISSR-2	6	1	16.6	202-685	0.03	0.18
SCoT- 3	14	6	42.8	175-1540	0.136	0.32	ISSR-4	21	6	28.5	147-364	0.09	0.32
SCoT- 4	6	0	0	284-799	0	0	ISSR-5	13	2	15.3	234-479	0.05	0.32
SCoT- 5	13	0	0	195-1347	0	0	ISSR-7	10	3	30	236-296	0.09	0.32
SCoT-12	15	7	46.6	153-855	0.195	0.42	ISSR-11	12	2	16.6	210-931	0.05	0.32
SCoT-13	11	3	27.2	168-1144	0.048	0.18	ISSR-12	8	2	25	174-946	0.04	0.18
SCoT-16	11	3	27.2	248-1594	0.048	0.18	ISSR-13	9	4	44.4	240-311	0.14	0.32
SCoT-20	13	7	53.8	252-1549	0.124	0.23	ISSR-14	14	5	35.7	120-374	0.11	0.32
SCoT-33	10	4	40	285-1520	0.128	0.32	ISSR-24	7	3	42.8	273-908	0.18	0.42
Total	115	34	275.9		0.775	1.97	Total	112	30	274.5		0.81	2.88
Mean	11.5	3.4	29.56		0.077	0.197	Mean	11.2	3	26.78		0.081	0.288

Note: Total amplified band (TAB), Polymorphic band no. (PBN), % polymorphic band (%PB), Band size/bp (BS/bp), Marker index (MI), Polymorphism information content (PIC).

Table 4. Genetic diversity summary for irradiated tomato Idkawy cultivar grew under salt stress revealed by SCoT and ISSR marker analysis

SCoT							
Genetic parameters	control	100Gy × 50mM	150Gy × 50mM	200Gy × 50mM	100Gy × 100mM	150Gy × 100mM	200Gy × 100mM
Effective no. of alleles (Ne)	1.8336 ±0.15	1.8395 ±0.16	1.8678 ±0.17	1.8677 ±0.17	1.8375 ±0.16	1.8326 ±0.17	1.85 ±0.16
Nei's genetic diversity (H)	0.4509 ±0.04	0.4516 ±0.05	0.4597 ±0.06	0.4593 ±0.06	0.4511 ±0.05	0.449 ±0.06	0.4549±0.05
Shannon's information index (I)	0.6424 ±0.05	0.6426 ±0.06	0.6509 ±0.06	0.6505 ±0.06	0.6421 ±0.06	0.6398 ±0.06	0.6461±0.06
ISSR							
	control	100Gy × 50mM	150Gy × 50mM	200Gy × 50mM	100Gy × 100mM	150Gy × 100mM	200Gy × 100mM
Effective no. of alleles (Ne)	1.6225 ±0.23	1.6016 ±0.231	1.6381 ±0.21	1.6221 ±0.24	1.6158 ±0.23	1.6074 ±0.22	1.6204 ±0.22
Nei's genetic diversity (H)	0.3712 ±0.09	0.3653 ±0.08	0.3792 ±0.08	0.3705 ±0.09	0.3687 ±0.09	0.3662 ±0.09	0.3715 ±0.09
Shannon's information index (I)	0.5541 ±0.10	0.5482 ±0.09	0.5636 ±0.09	0.5532 ±0.10	0.551 ±0.11	0.5484 ±0.10	0.5543 ±0.10

* Ne = Effective number of alleles [Kimura and Crow (1964)]; * H = Nei's (1973) gene diversity; * I = Shannon's Information index [Lewontin (1972)]

The polymorphic information content (PIC) values varied from 0 to 0.42, with an average of 0.197 (PIC < 0.5). The marker index (MI) values were at their lowest for SCoT-1, SCoT-4, and SCoT-5 (0.0), while the highest was recorded for SCoT-12 (0.195), with an average MI of 0.077, as detailed in Table 3.

ISSR Marker Analysis

A total of 112 bands were produced using ten ISSR primers, as depicted in Figure 6. Each of the bands displayed genetic diversity, with a range of 6 to 21 bands per primer and an average of 11.2 fragments per primer. The primer ISSR4 had the most bands (21), while ISSR1 had the fewest (6), averaging 11 bands per primer. The polymorphism percentage ranged from 15.3% (ISSR-5) to 44.4% (ISSR-13). The amplified products varied in size from 120 base pairs (ISSR17) to 946 base pairs (ISSR12). The polymorphic information content (PIC) values ranged from 0.18 (ISSR1, ISSR2, and ISSR12) to 0.42 (ISSR19), with an average of 0.28 (PIC < 0.5). The marker index value (MI) ranged from 0.03 (ISSR1 and ISSR2) to 0.18 (ISSR19), with an average of 0.081, as detailed in Table 3.

Genetic Diversity Revealed by SCoT and ISSR Markers

Table 4 summarizes the genetic diversity observed in tomato plantlets under different combined effects treatments. The SCoT marker analysis indicated that the effective number of alleles (Ne) ranged between 1.8326±0.18 (150Gy×100 mM) to 1.8678±0.17 (150Gy×50 mM). Likewise, Nei's genetic diversity (H) showed variation from 0.4490 ± 0.06 (150Gy×100 mM) to 0.4597± 0.06 (150Gy×50 mM). Additionally, the Shannon's information index (I) ranged from 0.6398±0.066 (150Gy×100mM) to 0.6509± 0.065 (150Gy×50mM). On the other hand, the ISSR marker revealed that the effective number of alleles (Ne) ranged from 1.6016± 0.22 (100 Gy× 50 mM) to 1.6381± 0.22 (150 GyX50 mM). The highest and lowest values of Nei's genetic diversity (H) were 0.3653±0.09 (100Gy×50mM) and 0.3792± 0.09 (150Gy×50mM), respectively. The Shannon's information index (I) ranged from 0.0563±0.1 (150 Gy× 50 mM) to 0.5543±0.11 (200 Gy× 100 mM). In general, the findings suggest that plantlets treated with 150 Gy × 100 mM showed the lowest genetic diversity values when analyzed with both SCoT and ISSR markers.

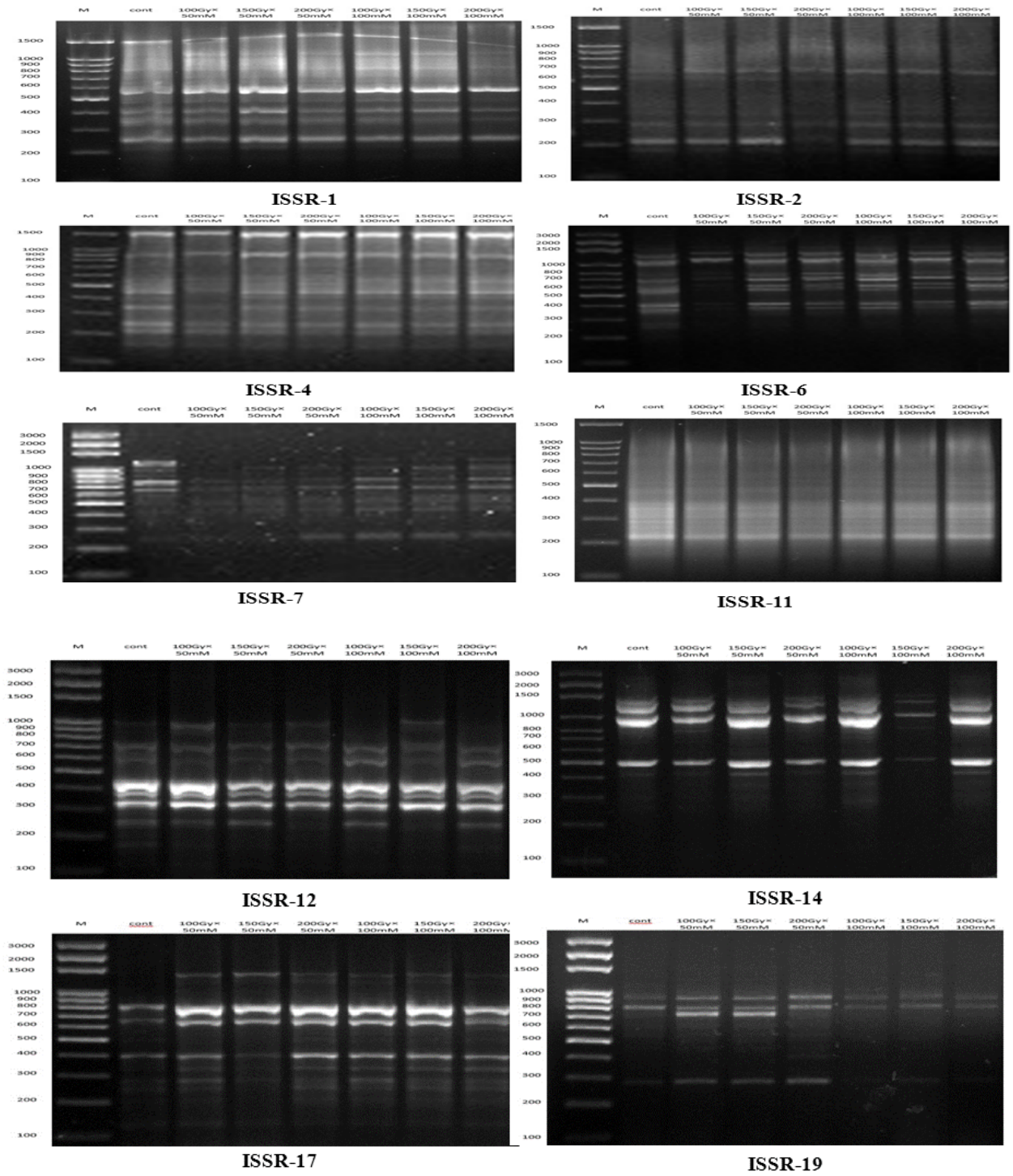

Figure 6. Representative ISSR profile of six combined effect between gamma radiation doses and salinity in tomato cv. Idkawy.

On the contrary, plantlets treated with 150 Gy × 50 mM and 100 Gy × 50 mM exhibited the highest diversity levels when examined with SCoT and ISSR markers, respectively.

Genetic Relationships

The study aimed to analyze the genetic relationships among irradiated tomato plantlets grew on salt stress by utilizing SCoT and ISSR markers.

SCoT Markers

The Jaccard distance coefficient was calculated to assess the genetic similarity between different treatments using binary scoring data from ten SCoT primers. The genetic distance ranged from 0.9036 to 0.9835. The smallest distance (0.9036) was observed between the untreated plantlets (control) and the treatment involving 150 Gy × 50 mM. On the other hand, the highest distance (0.9835) was found between the treatment involving 100 Gy × 50 mM and the treatment involving 200 Gy × 100 mM (refer to Table 5). To further analyze the similarity between the treatments, a cluster analysis was performed using Jacquard's similarity coefficients and the UPGMA algorithm. The resulting dendrogram (Figure 7) classified the combined effects treatments of tomato plantlets into three distinct groups.

Table 5. The genetic identity (above diagonal) and genetic distance (below diagonal) values among of irradiated tomato Idkawy cultivar grew under salt stress revealed by SCoT.

pop ID	Control	100Gy×50 mM	150Gy×50 mM	200Gy×50 mM	100Gy×100 mM	150Gy×100 mM	200Gy×100 mM
Control	-----	0.9164	0.9036	0.9383	0.9337	0.9697	0.916
100Gy×50mM	0.0873	-----	0.9305	0.9532	0.9457	0.9441	0.9835
150Gy×50mM	0.1014	0.072	-----	0.9343	0.9522	0.9231	0.9473
200Gy×50mM	0.0637	0.0479	0.068	-----	0.9255	0.9764	0.9507
100Gy×100mM	0.0686	0.0559	0.0489	0.0774	-----	0.9349	0.9569
150Gy×100mM	0.0308	0.0575	0.08	0.0239	0.0673	-----	0.942
200Gy×100mM	0.0878	0.0166	0.0542	0.0506	0.0441	0.0598	-----

Table 6. The genetic identity (above diagonal) and genetic distance (below diagonal) values among of irradiated tomato Idkawy cultivar grew under salt stress revealed by by ISSR.

pop ID	Control	100Gy×50 mM	150Gy×50 mM	200Gy×50 mM	100Gy×100 mM	150Gy×100 mM	200Gy×100 mM
Control	-----	0.9719	0.9981	0.9577	0.9977	0.9624	0.9954
100Gy×50mM	0.0285	-----	0.9739	0.9972	0.9747	0.9971	0.9699
150Gy×50mM	0.0019	0.0265	-----	0.9616	0.9985	0.9675	0.9978
200Gy×50mM	0.0433	0.0028	0.0391	-----	0.9621	0.998	0.9571
100Gy×100mM	0.0023	0.0256	0.0015	0.0386	-----	0.966	0.9991
150Gy×100mM	0.0384	0.0029	0.0331	0.002	0.0346	-----	0.9623
200Gy×100mM	0.0046	0.0306	0.0022	0.0438	0.0009	0.0384	-----

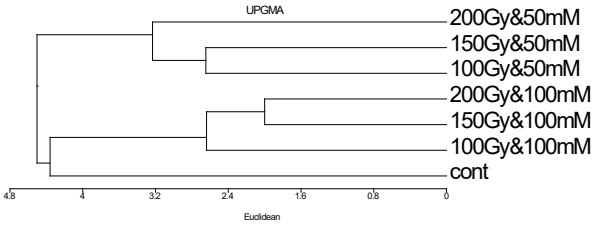

Figure 7. A dendrogram of six combined effect between gamma radiation doses and salinity in tomato cv. Idkawy revealed SCoT marker.

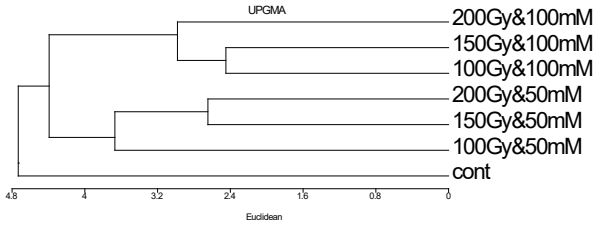

Figure 8. A dendrogram of six combined effect between gamma radiation doses and salinity in tomato cv. Idkawy revealed ISSR marker.

The first group comprised the treatments involving 200 Gy × 50 mM and 150 Gy × 50 mM, as well as 100 Gy × 50 mM. The second group included the treatments involving 200 Gy × 100 mM, 150 Gy × 100 mM, and 100 Gy × 100 mM. Lastly, the third group consisted of the untreated plantlets (control) and the treatments involving 200 Gy × 50 mM, 150 Gy × 50 mM, 100 Gy × 50 mM, 200 Gy ×100 mM, 150 Gy ×100 mM, and 100 Gy × 100 mM.

ISSR Markers

The Jaccard distance coefficient was computed between treatments using the binary scoring data obtained from the ten ISSR primers. The genetic distance ranged from 0.9577 to 0.9991. The smallest distance (0.9577) was observed between untreated plantlets (control) and the treatment with 200 Gy × 50 mM. On the other hand, the largest distance (0.9991) was found between the treatments with 100 Gy × 100 mM and 200 Gy × 100 mM (Table 6). To perform cluster analysis, Jacquard's similarity coefficients and the UPGMA algorithm were utilized. The resulting dendrogram (Figure 8) divided the combined effects treatments into three main groups. The first group comprised the treatments with 200 Gy × 100 mM and 150 Gy × 100 mM, as well as 100 Gy × 100 mM. The second group included the treatments with 200 Gy × 50 mM, 150 Gy × 50 mM, 100 Gy, and 50 mM. Lastly, the third group consisted of the untreated tomato plantlets (control) and the treatments with 200 Gy × 100 mM, 150 Gy × 100 mM, 100 Gy × 100 mM, 200 Gy × 50 mM, 150 Gy × 50 mM, and 100 Gy × 50 mM. Genetic markers, such as SCoT and ISSR markers, play a vital role in plant genetics and breeding by providing valuable insights into genetic diversity, population structure, and trait inheritance. SCoT markers have been successfully employed in various plant species, including crops, ornamentals, and medicinal plants, allowing researchers to assess genetic diversity and relatedness (Fahmy et al. 2020; El-Fiki et al. 2021). The high informativeness and reproducibility of SCoT markers make them suitable for studying population genetics, phylogenetics, and germplasm characterization (Collard and Mackill 2009; Xiong et al. 2011). ISSR markers have shown their worth as useful instruments in genetic research. These markers are known for their high level of polymorphism, making them well-suited for studying genetic diversity, fingerprinting, and marker-assisted selection (Gupta and Varshney, 2000; Bhattacharyya et al. 2013), have highlighted the importance of ISSR markers in genetic analysis. They have been widely employed to explore genetic diversity and population dynamics in various plant species. ISSR markers have contributed to our understanding of evolutionary relationships, identification of unique genotypes, and assessment of

genetic resources (Fahmy et al. 2020; El-Fiki et al. 2021). SCoT and ISSR markers are highly valued for their adaptability and simplicity. These markers are advantageous as they do not necessitate prior understanding of the genome and can be utilized for a wide range of plant species, whether they are commonly studied or not. They offer a quick and affordable way to assess genetic diversity and relationships. Additionally, SCoT and ISSR markers are PCR-based, allowing for efficient and high-throughput analysis of large numbers of samples. Their dominant nature simplifies scoring without the need for complex genotyping techniques.

Conclusion

The combined effect of gamma radiation and salinity stress on tomato plants grown in vitro presents a complex interaction that influences plant growth, development, and physiological responses. Understanding this combined stress is crucial for developing strategies to enhance plant tolerance and productivity in challenging environments. SCoT and ISSR markers are important genetic tools in plant genetics and breeding. Their versatility, simplicity, and cost-effectiveness make them valuable for studying genetic diversity, population structure, and trait inheritance.

Declarations

Acknowledgment

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Disclosure Statement

No potential conflicts of interest are reported by the authors.

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