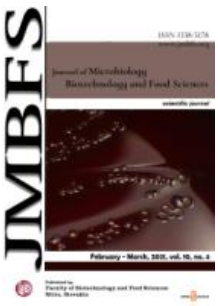

JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

THE GENETIC VARIATION ASSESSMENT OF IN VITRO IRRADIATED TOMATO (*Lycopersicon esculentum* Mill) BY SCoT AND ISSR MARKERS

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doi: 10.15414/jmbfs.2021.10.4.557-565

ARTICLE INFO

Received 4. 4. 2019
Revised 26. 9. 2019
Accepted 19. 10. 2019
Published 1. 2. 2021

Regular article

OPEN ACCESS

ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) are considered major and important globally vegetable crops and in Egypt in particular. Tissue culture techniques have encouraged the utilization of mutation methods in crop improvement. The mutation induction in vegetative crops through tissue culture may be the optimal method to improve these crops. Induced genetic variation in tomato plantlets by using gamma radiation and identified these changes through SCoT and ISSR markers. Egyptian tomato cultivar Idkawy explant was cultured onto MS medium supplemented with 0.2 mg^{-1} BAP. The resulted plantlets were irradiated with γ radiation doses (50, 100, 150, 200 or 250 Gy). The survival, growth rate, and mean of shoot length were decreased with increasing gamma radiation dose. The irradiated plantlets survival percentages were ranged from 78.75% to (50 Gy) and 18.75% to (250 Gy), whereas, the shoot length decreased by a rate of 2.71 cm for the dose (50 Gy) and 1.2 cm for dose (250 Gy). Genetic diversity was evaluated by SCoT and ISSR markers using ten primers for each. It was noticed that the polymorphism percentage mean of SCoT marker (60.53%) is higher than the ISSR marker (39.6). The *PIC* values average for both markers SCoT and ISSR were 0.429 and 0.347, as well, *MI* values were 0.345 and 0.156, respectively. On the other hand, the effective no. of alleles (*Ne*), Nei's genetic diversity (*H*) and Shannon's information index (*I*) parameters, it was found that the dose 100 Gy caused the highest genetic variation compared with other doses using SCoT marker, however, in ISSR marker was dose of 150 Gy the highest dose for induced genetic variation. 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. The relationships within treatments were assessed through cluster analysis (UPGMA) based on SCoT and ISSR analysis.

Keywords: DNA polymorphism, genetic variation, ISSR, radiation**,** SCoT, tissue culture, tomato

INTRODUCTION

Tomato is certainly considered one among the most significant vegetable yields all inclusive and Egypt specifically, where it's miles second one most significant vegetable harvests after potatoes. The world cultivated area is about 3.7 million hectares producing about 100 tons of fresh fruit. In Egypt, tomatoes are cultivated on nearly 3% of the all out cultivated area, with a growing season from summer to winter, http://www.fao.org/faostat/en/#data/QC. The *in vitro* techniques are perceived as valuable instruments in tomato improvement. The worth and significance of commercial tomato have been carried out many laboratory experiments *in vitro* to improve the crop through genetic manipulation **(Evans, 1989)**. It is common in plant tissue culture to use many hormones and growth regulators to result in adventitious shoots. Cytokinins is the most widely recognized and used to obtain adventitious shoots from tomato explants such as zeatin, thidiazorun (TDZ), kinetin (KIN) and 6-benzylaminopurine (BAP), **(El-Bakry, A.A., 2002; Moghaieb *et al*. 1999; Mohamed *et al*. 2010; Kalyani *et al*. 2014)**. Genotype and physiological status, as well as cytokinins and auxins concentrations and ratios, are also important factors affecting on adventitious shoots formation and plant regeneration in tomato (**Bhatia *et al*. 2004; Mamidala & Nanna, 2011; Kumar *et al*. 2017)**. The decrease of genetic diversity in tomato via domestication and breeding has brought about the requirement for conservation, characterization, and usage of genetic resources **(Terzopoulos & Bebeli, 2008).** Gamma radiation is ionizing radiation in which it reacts with atoms or molecules to provide free radical in cells. Gamma irradiation might actuate noteworthy morphological modifications in plant tissues in addition to a numerous of biochemical responses on the cellular level. Gamma radiation have given a high number of valuable mutants and is as yet indicating a raised potential for improving vegetative proliferated plants (Predieri 2001). Radicals may have a harmful effect on nucleic acid, carbohydrate and membrane lipids causing cell damage (Suzuki et al. 2012), or act on modifying the cell components and this impact may show up on the morphology, physiology, biochemistry, and anatomy depending on radiation doses. These impacts incorporate changes for the plant cell structure and metabolism, e. g. dilation of thylakoid membranes, modification in photosynthesis, balance of the antioxidative system and accumulation of phenolic compounds **(Kim et al., 2004; Wi *et al*., 2005)**. The utilize of molecular markers not only depends on the appraisal of germplasm collections genetic variation but also on the distinction between the genotypes of populations. The SCoT marker technique depends on the single primer amplified region precept in which it makes use of a single primer as a forward and reverse primer, proven dominant markers just like the RAPD or ISSR technique. However, the usage of PCR amplification to SCoT primers objectives gene regions surrounding the ATG inception codon on each DNA strands. The SCoT markers are anticipated upon to be linked to functional genes and corresponding traits, as a result the amplicons may be converted to gene-targeted marker systems **(Xiong *et al*., 2011).** Generally, SCoT markers were reproducible but the factors determining reproducibility as primer length and annealing temperature are not the only factors **(Collard & Mackill, 2009).** Inter simple sequence repeat (ISSR) marker is additionally relies upon PCR method. This technique relies upon on amplifying a segment of DNA this is at a distance that may be located among two identical microsatellite repeat regions oriented in contrary directions. Usually the ISSR technique use single primer with lengthy ranged from 16- 25 bp, the PCR reaction targeting multiple genomic loci to amplify distinctive amplicons. Microsatellite repeats primers have been used may be di-nucleotide, tri-nucleotide, tetra-nucleotide or penta-nucleotide and either unanchored **(Gupta *et al*., 1994; Meyer *et al*., 1993; Wu *et al*., 1994; Ng & Tan, 2015)** or anchored at ′3 or ′5 end with one to four degenerate bases extended into the flanking sequences **(Zietkiewicz *et al*., 1994).** The aim of this study is to investigate the effect of some hormones and growth regulators on plant regeneration, determine the impact of gamma radiation doses on plantlets growth and survival, as well the mutagen of gamma radiation induced morphological and genetical variations, and evaluate the effectiveness of SCoT

and ISSR markers for genetic variation analysis in tomato plantlets (*Lycopersicon esculentum* Mill.) Idkawy.

MATERIAL AND METHODS

Seed Material

Tomato Seeds, Idkawy cultivar were obtained from Agricultural Research Centre, Vegetable Research Institute, Ministry of Agriculture, Egypt**.**
Seeds were sterilized by dipping in Clorox (30%) for ten minutes followed by three rinses in sterile distilled water. The seeds have been cultured on solid MS medium **Murashige & Skoog (1962)** hormone free. The propagation process began after six to eight weeks when the length of the plantlets was 12 cm. The culture was maintained through cutting into single nodes. The culture medium supplemented with different concentrations of hormones has been tested as, NAA, KIN, IAA, IBA or/ and BAP Table 1. A further 100 segments were cultured onto MS medium on each hormone. The pH of the culture medium was adjusted to 5.7 before autoclaving and the buds were thereafter incubated in the growth chamber at 25 °C ± 2 under photoperiod 16 h.

Table (1) Effect of some growth regulator on tomato plantlets growth supplemented to MS medium.

Medium No.	growth regulators	Plantlet Formation	Callus formation	Shoot formation	Root formation
1	$8mg^{-1}$ NAA+ 0.01mg/L KIN	+	+	+	-
2	0.5mg/L IAA	++	-	+	+
3	MS	+++	-	+	+
4	5mg/L BAP	-	-	+	-
5	0.1mg/L IBA	+++	-	+	+
6	0.2 mg/L BAP	++++	-	+	+

(+) = weak, (++) = medium, (++++) = High

Gamma irradiation

Irradiation was carried out with the ^{137}Cs source at the dose rate 1 Gy/ 2 min 30 sec, at National Centre for Radiation Research and Technology, Cairo, Egypt.
Tomato seeds soaked in water and exposed to distinct gamma irradiation doses (50, 100, 150, 200 and 250 Gy). An in addition 80 seeds had been irradiated with gamma rays at every dose. The irradiated seeds had been sterilized by dipping in Clorox (30%) for ten mins accompanied by 3 rinses in sterile distilled water. The seeds had been cultured on solid MS medium **Murashige & Skoog (1962)** hormone free. Micropropagation started out after 6- eight weeks whilst the plantlets had been approximately 10-12 cm high. The culture was maintained by cutting into single nodes and transferring them onto MS medium supplemented with 0.2 mg/L BAP. The pH of the culture medium was adjusted to 5.7 before autoclaving and the buds had been thereafter incubated in the growth chamber at 25 °C ± 2 under photoperiod 16 h.

Genomic DNA Extraction

Total genomic DNA was isolated from about two grams of irradiated plantlets and grew on MS medium supplemented with 0.2 mg $^{-1}$ BAP according to the protocol described by **Anderson** *et al***. (1992)** with a few modifications intended to improve the quality of DNA: two consecutive extractions with phenol: chloroform (1:1) were carried out by an additional wash of 97% alcohol (left at -20 °C for one hour) an 70% pre-cooled ethanol, respectively **El-Fiki & Adly (2019)**. The yield and quality of DNA had been assessed by gel electrophoreses.

SCoT – PCR amplification

Ten (SCoT) primers had been selected according to **Collard & Mackill (2009)**, (Table 2). Amplification reactions had been achieved in a total volume of 25 µl, which contained 250 µM of every primer, 0.2 mM of every deoxynucleotide, 1.5 mM $MgCl_2$, 1 unit *Taq* polymerase, and 50- 100 ng of template DNA. All reaction volumes had been 25 µl overlaid with a drop of mineral oil. The thermocycling program used was: one cycle at 94 °C for 3 min, 35 cycles at 94 °C for 50 sec,1 min at 50 °C, 2 min at 72 °C, and the final extension step of 7 min at 72 °C. Electrophoresis was done to visualize the PCR amplified product. It was carried out on 1.0% agarose gel and amplified fragments had been visualized by staining with ethidium bromide **(Ezzat et al. 2019)**.

ISSR – PCR amplification

Ten different ISSR primers which have been selected are eleven or 18 nucleotides based on di-, tri- or tetra-nucleotide SSR repeats with 2 nucleotides ′3 selective anchor 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 $GAC(GATA)_4$ Table 2. Amplification reactions had been completed in a 25 µl volume, containing: 20 mM Tris-HCl (pH 8.4), 50mM KCl, 2.5 mM MgCl2, 200 µM each of dNTPs, 1 µM primer, 30 ng of genomic DNA 1.5 U of *Taq* DNA polymerase. The reaction mixture was overlaid with two drops of mineral oil, incubated for 3 min. at 95 °C for initial denaturation, and then amplified for 45 cycles consisting of the 30s at 94 °C, 30s at 45 °C and 60s at 72 °C followed by 7 min. incubation at 72 °C. Amplification products were separated by gel electrophoresis on precast 0.8% agarose and visualized under UV illumination after staining with ethidium bromide and photographed **(El-Fiki et al. 2017)**.

Data analysis

Fragment sizes of each SCoT and ISSR had been decided with PyElph 1.4 software **(Pavel & Vasile 2012)** comparison with the DNA marker. Amplified products had been scored as present (1) or absent (0) to form a binary matrix.
So as to quantify the informativeness of the markers to distinguish among genotypes, polymorphism information content (PIC) and marker index (MI) had been calculated. PIC was calculated consistent with to the formula of **Anderson** ***et al.*** **(1992)**, as PIC = 1- Σp_i^2 where *pi* is the frequency of the *i*th allele of the locus in six gamma radiation treatments. MI was decided according to **Varshney** ***et al.*** **(2007)**, because the fabricated from PIC and effective multiplex ratio. To characterize genetic variation, a few parameters, which includes the effective number of alleles (*Ne*), Nei's gene diversity (*H*) and

Table 2 Primers code and nucleotide sequences of the ten used SCoT and ISSR primers.

SCoT				ISSR		
N0.	**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

Shannon's information index (*I*) had been calculated the usage of PopGen 1.3.1 software, **(Yeh *et al*., 1990)**. Jaccard's similarity coefficient was calculated to assemble a similarity matrix and the UPGMA algorithm was used to carry out hierarchical cluster analysis and to assemble a dendrogram the usage MVSP, Ver 3. 1 **Kovach, (1998)**.

RESULTS AND DISCUSSION

Propagation of tomato *in vitro*

For the selection of most suitable hormone for the growth of tomato plantlets, the explants had been cultured on MS medium supplemented with numerous hormones concentrations which include KIN, IAA, 6-BAP or/ and IBA**.** The impact of those hormones was the formation of callus, the difference in plantlets growth rates, the formation of plantlets without roots or intact plantlets proven with inside (Table 1). Based on those results, BAP at concentration 0.2 mg^{-l} was the hormone that generated growth of tomato plantlets as proven in Table 1. Therefore, MS medium supplemented with 0.2 mg^{-l} BAP was used for additional radiation experiments. For tomato regeneration, a huge assortment of plant growth regulators has been utilized at different concentrations. High shoot regeneration observed in different tomato cultivars when cotyledons, stems, leaves and hypocotyl cultured on MS medium supplemented with 5.0 µM BAP or 1.0 mg-l zeatin (**Pino *et al*., 2010; Godishala *et al*., 2012; Arkita *et al*., 2013**). However, **Bookout and Noble (1987)** found that the supplemented IAA to MS medium decrease regeneration and enhance shoot induction by 20%.

Effect of gamma radiation on tomato survival plantlets

Plantlets of tomatoes had been irradiated with numerous gamma radiation doses (0, 50, 100, 150, 200 or 250 Gy). The survival of irradiated plantlets was reduced with increasing gamma radiation doses as illustrated in Fig 1. The number of plantlets survival percentages ranged from 78.75% (50 Gy) to 18.75% (250 Gy). As well the mean of plantlets shoot length was decreased with increasing gamma radiation doses as shown in Table 3, where the shoot length decreased by a rate of 2.71 cm for the dose 50 Gy and 1.2 cm for dose 250 Gy. Plant regeneration may be acquired directly **(Dwivedi *et al*., 1990)**, or indirectly via callus **(Jawahar *et al*., 1997)**. An extensive variety of plant growth regulators at different concentrations have been utilized alongside various explants for various cultivars of tomato in different examinations for induction of callus and plant regeneration. The specific action of growth regulator depended both on genotype and physiological condition of the donor plant. A similar tendency of phytohormone impact on organogenesis was likewise seen by **(Dewi *et al*., 2004; Miceska, 2011)**. Gamma ray is ionizing radiation react with atoms or molecules to provide free radical in cells. Radicals may also have a damage impact or act on rearranging the cell components and this impact may also seem at the morphology, physiology, biochemistry, and anatomy relying on radiation doses. These impacts include modifications with inside the plant cellular structure and metabolism, e. g. dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds **(Kim et al., 2004; Wi *et al*., 2005)**. Chronic exposure has to a great extent been utilized however doesn't seem to have any favorable circumstances over intense irradiation **(Sigurbjörnsson, 1977)**, which is progressively reasonable for induced mutagenesis in tissue cultures. Generally, gamma irradiation may be used to achieve varieties which are economically essential in agriculture, with excessive productiveness and quality **(Jain, 2010; El-Fiki *et al*., 2018)**. Mutations are of paramount significance to be used in breeding programs and *in vitro* mutagenesis in order to increase the genetic diversity required for plant improvement programs. Many mutant varieties have been developed, which are resistant to biotic and abiotic stress and with excessive quality **(Jain *et al*., 2013)**. Several tries of mutagenic treatment on cultured anthers were mentioned in higher plants **(Sangwan & Sangwan, 1986; MacDonald *et al*., 1988; Ling *et al*., 1991; El-Fiki *et al*., 2015)**. These results had been acquired according with the radiation affectability test is done by **Hasegawa *et al*. (1995), El-Fiki *et al*. (2015, 2016)** for tobacco, **(El-Fiki *et al*., 2018)** for potato, **El-Fiki *et al*. (2005a, b)** for alfalfa, **Norfadzrin *et al*. (2007)** for tomato and okra and *Orthosiphon stamineus* **Kiong *et al*. (2008)**.

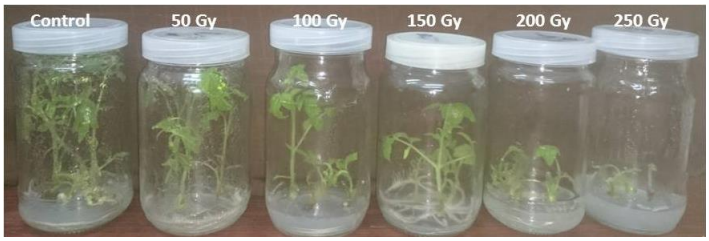


Figure 1 Gamma radiation doses impact on the survival of tomato plantlets

Table 3 Gamma irradiation doses impact on bud survival in tomato plantlet.

γ-Radiation dose /Gy	No. of growing plantlets	%Bud survival	Mean of shoot length (cm)
Control	74	92.5	5.30
50	63	78.75	2.71
100	59	73.75	2.30
150	40	50	2.00
200	32	40	1.54
250	15	18.75	1.20

Molecular Markers

The polymorphism of amplified products from irradiated tomato

SCot analysis

Total genomic DNA from irradiated tomato plantlets (*Lycopersicon esculentum* Mill.) with diverse gamma irradiation doses 0, 50, 100, 150, 200 and 250 Gy have been used as templates for SCoT and ISSR genetic diversity analysis. The SCoT and ISSR analysis among irradiated plantlets are summarized in Table 4.

Table 4 Amplification consequences generated through 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	13	9	69.23	137-686	0.42	0.50	ISSR-1	17	11	64.7	163-1553	0.29	0.42
SCoT- 2	10	6	60.0	277-1166	0.18	0.23	ISSR-2	3	0	0.00	171-253	0.14	0.42
SCoT- 3	14	7	50.0	258-1302	0.42	0.50	ISSR-4	9	4	44.4	169-470	0.13	0.23
SCoT- 4	4	2	50.0	283-484	0.21	0.42	ISSR-5	13	6	46.1	142-1046	0.22	0.42
SCoT- 5	18	12	66.67	161-1426	0.41	0.50	ISSR-7	10	3	30.0	175-743	0.13	0.32
SCoT-12	15	13	86.67	172-747	0.46	0.50	ISSR-11	11	7	63.6	206-891	0.26	0.42
SCoT-13	12	9	75.0	159-1174	0.45	0.50	ISSR-12	11	3	27.2	183-1052	0.14	0.32
SCoT-16	5	1	20.0	133-684	0.06	0.32	ISSR-13	7	3	42.8	232-1038	0.18	0.42
SCoT-20	8	3	37.5	285-953	0.37	0.50	ISSR-14	12	3	25.0	219-1682	0.04	0.18
SCoT-33	15	7	46.67	255-1463	0.47	0.32	ISSR-24	8	0	0.00	169-836	0.03	0.32
Total	114	69			3.45	4.29	Total	101	40			1.56	3.47
Mean	11.4	6.9	60.53		0.345	0.429	Mean	10.1	4	39.6		0.156	0.347

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

Ten ScoT primers amplified (Fig. 2) a total 114 amplicons with a range of 4 to 18 bands per primer with an average 11.4 fragments per primer. The highest numbers of bands (13) were generated by primer SCoT- 5, whereas the lowest number of bands (1) was generated by primer SCoT- 4 with an average 6.9 band per primer. The polymorphism varied from 20% to 86.67% with an average polymorphism of 60.53%. The size of the amplified products ranged from 133 bp (SCoT- 16) to 1463 bp (SCoT- 33). The polymorphic Information content values (*PIC*) were varied from 0. 23 (SCoT- 2) to 0.50 (SCoT- 1, 3, 5, 12, 13and 20)

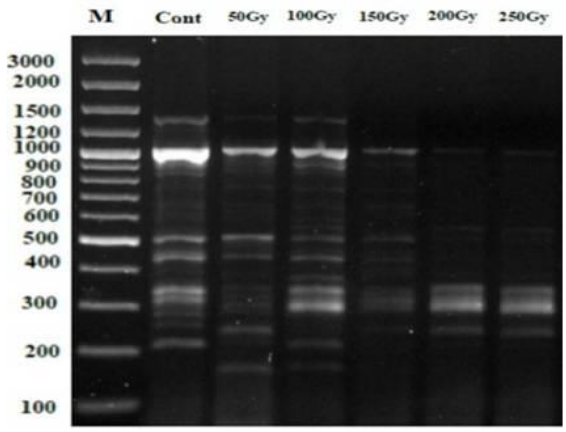
with an average 0.429 (*PIC* <0.5). Marker index (*MI*) value, the highest value showed for SCot- 33 (0.47), while the lowest value observed to SCoT- 16 (0.06) with an average 0.345 Table 4.

ISSR analysis

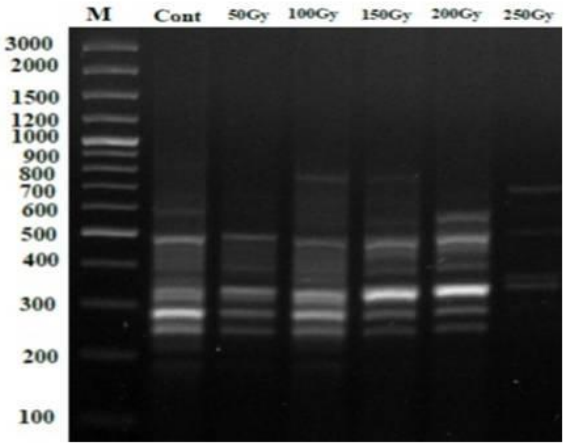
A total of 101 bands were generated from ten ISSR primers (Fig. 3) and all of them are polymorphic bands by a range of 3 to 17 bands per primer with an average of 10.1 fragments per primer. The highest number of bands 11 observed by ISSR- 1, while the lowest number was zero by ISSR- 2 and ISSR- 24 with an average of 4 bands per primer. The polymorphism percentage varied from 0% to 64.7%, with the mean of polymorphism 39.6%. The size of the amplified products ranged from 142 bp (ISSR- 5) to 1682 bp (ISSR- 14). The value of polymorphic information content (*PIC*) was varied from 0.18 (ISSR- 14) to 0.42 (ISSR- 1, 2, 5, 11 and 13) with an average 0.347 (*PIC* <0.5). Marker index value (*MI*) was varied between 0.03 (ISSR- 24) and 0.29 (ISSR- 1) by an average 0.156 Table 4.

Genetic diversity revealed by SCoT and ISSR markers

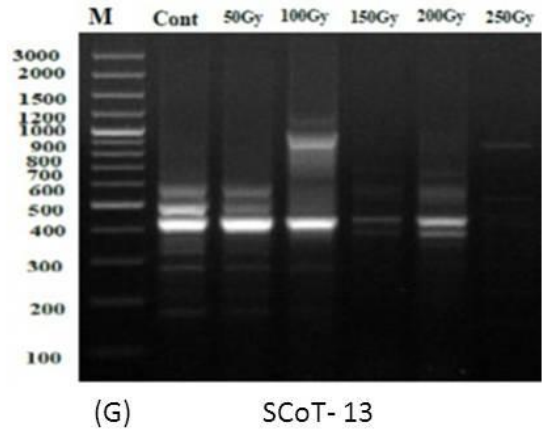
The genetic diversity between irradiated tomato plantlets with different gamma irradiation doses 0, 50, 100, 150, 200 or 250 Gy is summarize in Table 5. For the SCoT marker, the effective number of alleles (Ne) value ranged from 1.3633±0.24 (200 Gy) to 1.6478±0.28 (100 Gy). Whereas, Nei's genetic diversity (*H*) varied from 0.2499±0.10 (200 Gy) to 0.3758±0.11 (100 Gy). Also, the Shannon's information index (*I*) ranged from 0.4097±0.12 (200 Gy) to 0.5574±0.12 (100 Gy). It is noticeable in these results that the highest and least differences were found in irradiated plantlets with doses 100 and 200 Gy, respectively. On the other hand, the genetic diversity assessment by ISSR marker revealed an effective number of alleles (Ne) ranging from 1.6516±0.22 (200 Gy) to 1.7687±0.17 (0 Gy), as well as the highest and lowest value of Nei's genetic diversity (*H*) was 0.4295±0.05 (0 Gy) and 0.3849±0.08 (200 Gy) respectively. Furthor, Shannon's information index (*I*), was highest and lowest at non-irradiated plantlets (0 Gy) and (200 Gy) with 0.6198±0.05 and 0.5704±0.08 respectively. Of the results obtained, we note that the lowest genetic diversity values in both SCoT and ISSR analyzes was seen in plantlets irradiated at dose 200 Gy, while the highest value was the non-irradiated plantlets in ISSR marker and plantlets irradiated with dose 100 Gy.



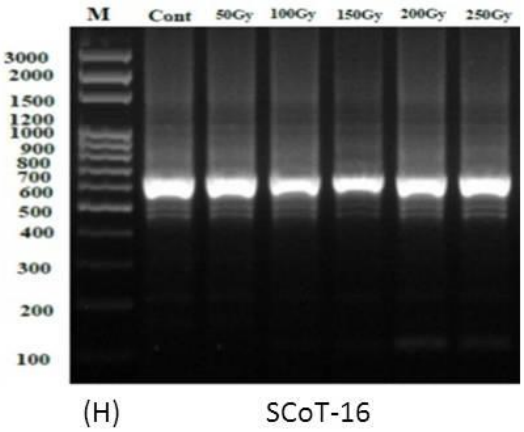
(E) SCoT- 5



(F) SCoT- 12



(G) SCoT- 13



(H) SCoT-16

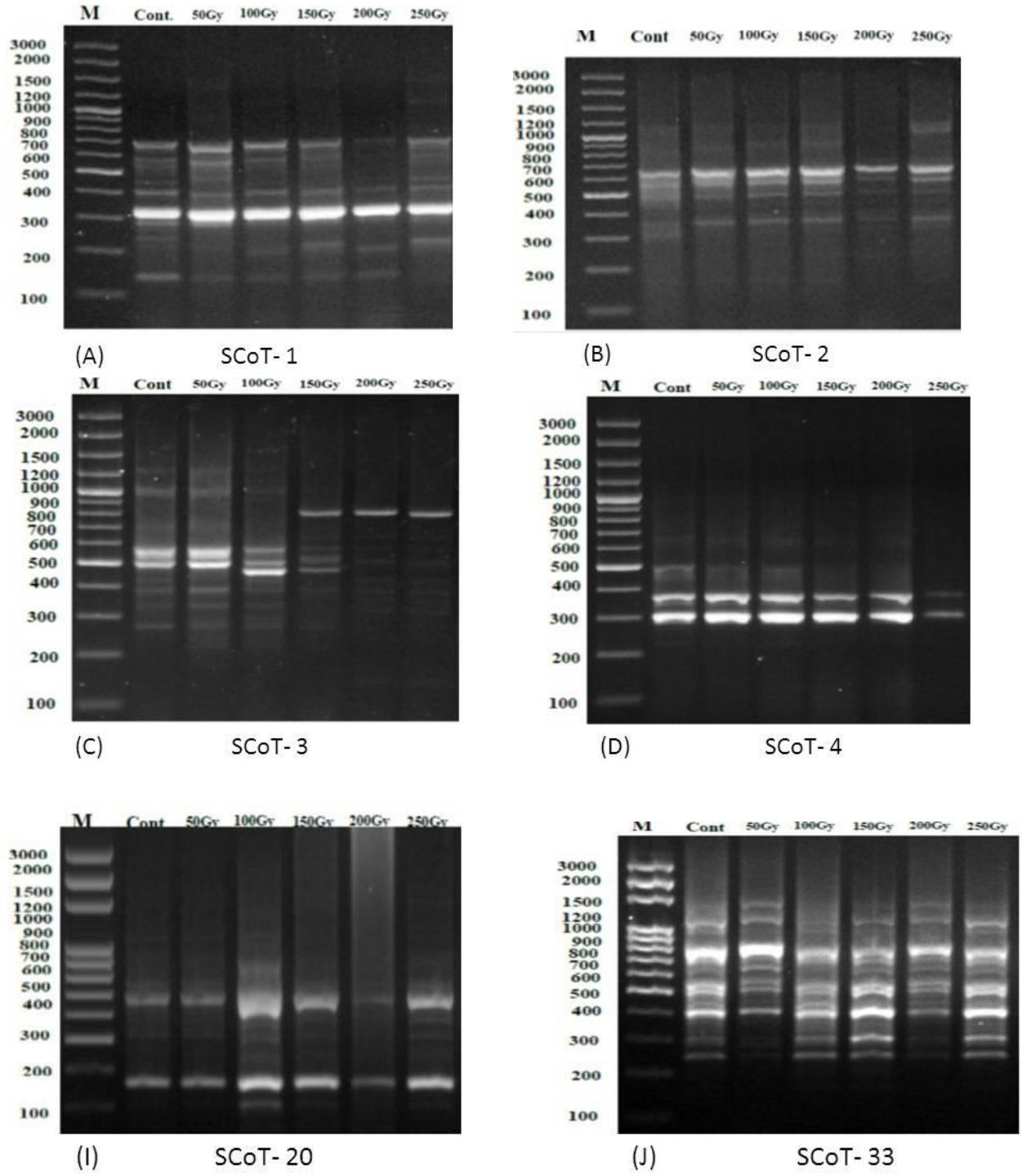


Figure 2 Representative of ten SCoT primers profile of irradiated tomato plantlets. Lane (1) Control (0 Gy); Lane (2) 50 Gy; Lane (3) 100 Gy; Lane (4) 150 Gy; Lane (5) 200 Gy; Lane (6) 250Gy.

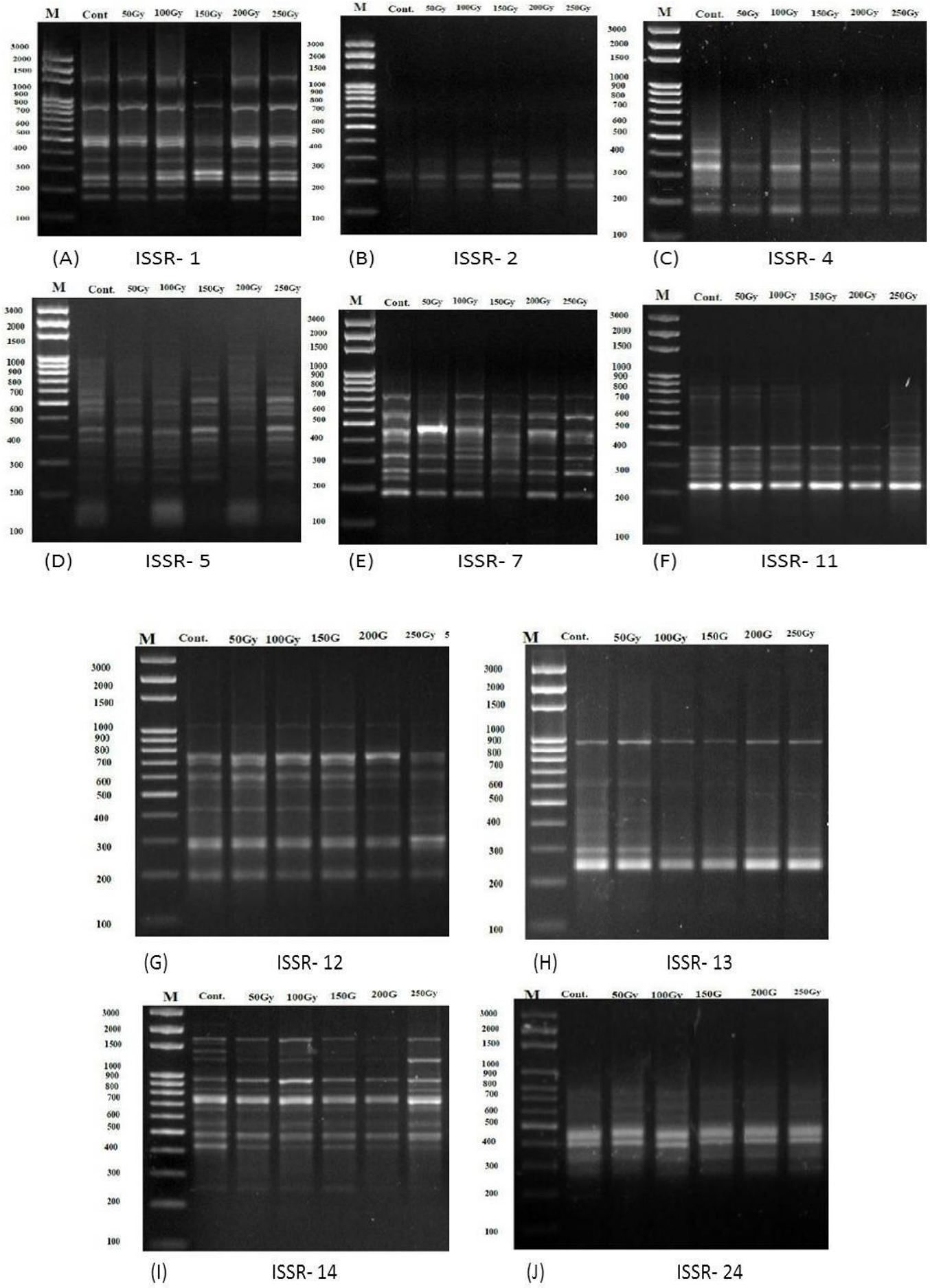


Figure 3 Representative of ten ISSR primers profile of irradiated tomato plantlets. Lane (1) Control (0 Gy); Lane (2) 50 Gy; Lane (3) 100 Gy; Lane (4) 150 Gy; Lane (5) 200 Gy; Lane (6) 250Gy.

Table 5 Genetic diversity summary between irradiated tomato plantlets with doses (0, 50, 100, 150, 200 or 250 Gy) revealed by SCoT and ISSR marker analysis

Genetic parameters	Marker											
	SCoT						ISSR					
	0 Gy	50 Gy	100 Gy	150 Gy	200 Gy	250 Gy	0 Gy	50 Gy	100 Gy	150 Gy	200 Gy	250 Gy
Effective no. of alleles (Ne)	1.5968 ±0.24	1.5328 ±0.23	1.6478 ±0.28	1.4691 ±0.23	1.3633 ±0.24	1.3756 ±0.23	1.7687 ±0.17	1.7360 ±0.23	1.6478 ±0.28	1.7419 ±0.21	1.6516 ±0.22	1.7629 ±0.24
Nei's genetic diversity (H)	0.3592 ±0.10	0.3323 ±0.11	0.3758 ±0.11	0.3046 ±0.10	0.2499 ±0.10	0.2569 ±0.10	0.4295 ±0.05	0.4136 ±0.08	0.4205 ±0.08	0.4166 ±0.08	0.3849 ±0.08	0.4217 ±0.08
Shannon's information index (I)	0.5391 ±0.12	0.5075 ±0.13	0.5574 ±0.12	0.4766 ±0.12	0.4097 ±0.12	0.4171 ±0.13	0.6198 ±0.05	0.6013 ±0.09	0.6082 ±0.09	0.6043 ±0.09	0.5704 ±0.08	0.6096 ±0.09

Table 6 Specific markers generated by SCoT and ISSR primers in irradiated tomato plantlets.

SCoT		ISSR	
Primer No.	Specific markers	Primer No.	Specific markers
SCoT -1	219 bp	ISSR- 1	878-439-273-231-207 bp
SCoT -2	277 bp	ISSR- 2	0
SCoT -3	1026-559-483-476 bp	ISSR- 4	470
SCoT -4	0	ISSR- 5	0
SCoT -5	259-231 bp	ISSR- 7	0
SCoT -12	0	ISSR- 11	0
SCoT -13	0	ISSR- 12	0
SCoT -16	0	ISSR- 13	0
SCoT -20	0	ISSR- 14	0
SCoT -33	1179-1439-704-547-420 bp	ISSR- 24	169 bp

Specific markers of irradiated tomato plantlets

Specific genetic markers were obtained from tomato plantlets irradiated with gamma rays by both SCoT and ISSR primers are summarized in Table 6. Five out of ten SCoT primers tested with irradiated tomato plantlets were successfully to generate specific markers (SCoT- 1, SCoT- 2, SCoT- 3, SCoT- 5 and SCoT- 33) varying from 219 bp (SCoT- 1) to 1439 bp (SCoT- 33). The SCoT specific markers ranged from one band (SCoT- 1 and SCoT- 2) to five bands by SCoT- 33. On the other hand, of the ISSR primers were screened, seven of the ten primers did not generate specific markers. ISSR- 1, 4 and 24 primers produced specific markers varied from [169 bp (ISSR- 24) to 878 bp (ISSR- 1)]. The highest specific markers were five bands observed with ISSR- 1, while the two other primers generate one band.

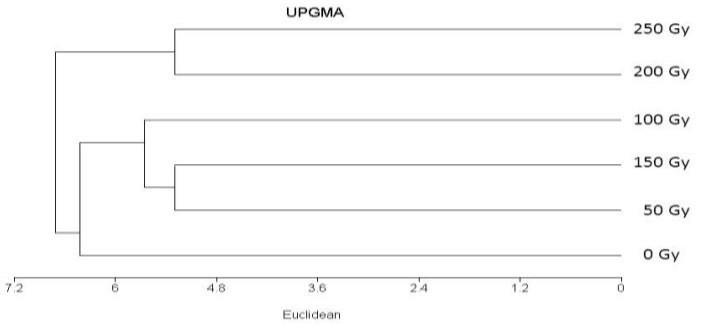


Figure 4 A dendrogram showing the genetic distance among six irradiated tomato plantlets using SCoT data

Genetic relationships

The genetic relationships between five gamma radiation treatments and non-irradiated tomato plantlets through SCoT and ISSR markers were investigated. Cluster analysis based on Jacquard's similarity coefficients and UPGMA algorithm was calculated. According to both marker ScoT and ISSR dendrogram, the six irradiated tomatoes classified into two groups. Based on SCoT dendrogram, the first group consists of irradiated tomato with doses 50, 150 and 100 Gy. However, the second group containing tomato plantlets irradiated with 200 and 250 Gy Fig. 4. On the other side, ISSR dendrogram, the first group consists of tomato plantlets irradiated with doses 50 and 250 Gy, while the second group consists of 100, 150 and 200 Gy Fig. 5.

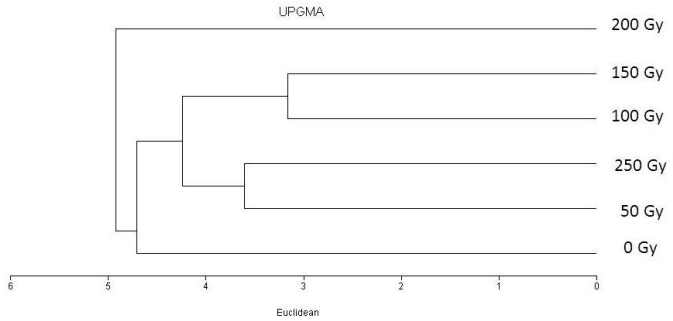


Figure 5 A dendrogram showing the genetic distance among six irradiated tomato plantlets using ISSR data

The efficacy of SCoT and ISSR genetic markers is evaluated via unique criteria such a *PIC* and *MI*, which were utilized in different research to evaluate exclusive germplasm and cultivated genotypes **(Gomes *et al*., 2009; Grativol *et al*., 2011; Patra *et al*., 2008; Tatikonda *et al*., 2009)**. The results received imply that, the each of *PIC* and *MI* values will be attributed investigate of genetic diversity. The amplicons number, *PIC* and *MI* of the SCoT and ISSR markers observed in this study is comparable to the results acquired through in tomato **(Shahlae *et al*., 2014; Abdein *et al*., 2018)**, durum wheat **(Etminan *et al*., 2016)**, *Dendrobium nobile* **(Bhattacharyya *et al*., 2013)**, groundnut **(Xiong *et al*., 2011)**, mango and **(Luo *et al*., 2010)**. Nowadays, SCoT marker has been successfully used since its discovery by **Collard & Mackill (2009)** in the assessment and analysis of the genetic diversity **(Fang-Yong *et al*., 2014; Jiang *et al*., 2014; Satya *et al*., 2015; Zhang *et al*., 2015)**, where it proved successful and efficient in the evaluation and analysis of the genetic diversity in order to contain high reproducibility and great power for the detection of polymorphism **(Galvan *et al*., 2003; Cao *et al*., 2006; Sofalian *et al*., 2009; Guo *et al*., 2012; Hamidi *et al*., 2014)**. On the other side as well, the ISSR technique is defined as regions in the genome flanked with microsatellite frequencies. These regions through the use of single primers and PCR amplification result from multiple amplification products which can be used to determine genetic variation in most organisms as a dominant multilocus marker. Consequently, this technique is considered to be the easiest and most reliable technique to be compared to other techniques such as RAPD, AFLP, and RLFP (**Wang *et al*. 2012; Shafiei-Astani *et al*. 2015; Ng & Tan, 2015**). ISSRs have been effectively used to appraise the extent of genetic variation at inter and intraspecific level in a wide range of crop species which include tomato. Primers of ISSR with (AG), (GA), and (CT), (TC), (AC), (CA) repeats show a higher polymorphism than those with other di-, tri- or tetra-nucleotide repeats. AT repeats are the most abundant di-nucleotides in plants. The frequency of Tri and tetra-nucleotides and their use are less di-nucleotides. (**Shahlaei *et al*., 2014; *Metwali et al*., 2016; Etminan *et al*., 2016; Abdein *et al*., 2018)**. In General, the difference between both analyzes (SCoT

and ISSR) is that each of them with targets on different parts of the genome **(Gajeraa *et al*., 2010)**.

CONCLUSION

The use of plant tissue culture techniques has been instrumental in developing, facilitating and overcoming many agricultural problems, including the limitations in the application of mutation techniques. Gamma ray radiation as a physical mutagenic is considered to be the safest and cheapest ways to induce genetic changes in the plant, especially in tissue culture where it is easy to identify and limit these changes accurately and easily. The study proved that both SCoT and ISSR were very successful in detecting genetic changes induced by gamma irradiation. SCoT marker was more accurate and efficient than ISSR marker for identification and genetic diversity analysis of irradiated tomato plantlets.

Acknowledgment: This research was carried out in National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority.

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