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GENETIC DIVERSITY ASSESSMENT OF *IN VITRO* IRRADIATED TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) USING SCOT MARKERS

[48]

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is considered the major and important globally vegetable crops especially in Egypt. Tissue culture techniques have facilitated the induction of mutant which helps in crop improvement. The mutation induction in vegetative crops through tissue culture may be the optimal method to improve these crops. Tomato explants of Idkawy Egyptian cultivar were cultured *in vitro* on MS medium supplemented with 0.2 mg/L BAP. The resulted plantlets were irradiated with different gamma radiation doses (50, 100, 150, 200 or 250 Gy) and the survival and mean of shoot length decreased as gamma radiation doses increased. The survival percentages of irradiated plantlets were ranged from 78.75% with 50 Gy dose to 18.75% with 250 Gy dose, while the shoot length values were decreased by a rate of 2.71 cm for dose 50 Gy and 1.2 cm for 250 Gy dose. The ten SCoT primers amplified a total of 114 amplicons with a range from 4 with SCoT-4 primer to 18 amplicons with SCoT-5 primer with an average of 11.4 amplicons per primer, The radiation specific markers were ranged from one fragment with SCoT-1 and SCoT-2 primers, SCoT-5 two fragments with primer to five fragments with SCoT-3 and SCoT-33 primers.

Keywords: Tomato, Idkawy cultivar, tissue culture, radiation, SCoT markers, DNA polymorphism.

INTRODUCTION

Tomato *(Lycopersicon esculentum* Mill.) is the second most important vegetable crop next to potato. Present world production is about 100 million tons fresh fruit from 3.7 million hectare. In Egypt, tomatoes are cultivated on about 3% of total cultivated area and grown in the three seasons; winter, summer and autumn (**FAO, 2016).** Plant tissue culture techniques are recognized as useful instruments in tomato improvement. Many different applied experiments were conducted on tomatoes *in vitro* for their commercial value of the crop and its amenability for further improvement via genetic manipulation **(Evans, 1989).** Several studies have been conducted on plant regeneration from a wide range of tissues and organs of wild and cultivated tomato germplasm **(Cassells 1979, Zapata et al 1981).** Electromagnetic radiations are of various types such as gamma rays, X-rays, visible light, and UV rays **(Wi et al 2005)**. Gamma rays are ionizing rays that react with atoms and molecules present inside the cells to produce free radicals. Production of free radicals depends on the irradiation level that causes damage or modification of components in plants, ultimately affecting morphology, physiology, anatomy, and biochemistry of plants **(Ali et al 2016).**

The SCoT markers have been successfully used to evaluate genetic diversity and structure, identify cultivars, and for quantitative trait loci (QTL) mapping and DNA fingerprinting in different species, including wheat, rice, check pea, sugar-

cane and grape **(Collard & Mackill 2009 and Que et al 2014).**

The objectives of this study were to examine the gamma irradiation impact on survival and growth of tomato plantlets (*Lycopersicon esculentum* Mill.) for Idkawy cultivar and the assessment of start codon targeted (SCoT) markers for analysis of the genetic diversity amang the control and irradiated plants.

MATERIALS AND METHODS

Materials

Tomato seeds of Idkawy cultivar were obtained from Vegetable Research Institute, Agricultural Research Centre, Giza, Egypt**.**

Methods

Seeds were sterilized by dipping in Clorox (30%) for ten minutes followed by three rinses in sterile distilled water. The seeds were cultured on solid MS medium (**Murashige and Skoog 1962)** without any hormone. Micropropagation was done after 6-8 weeks when the plantlets were about 10-12 cm in high. The culture was maintained by cutting it into single nodes. The cultured MS medium was supplemented with different hormone types and concentrations as shown in **Table (1)**. The pH of the culture media was adjusted to 5.7 before autoclaving them and the buds were thereafter incubated in agrowth chamber at 25 °C ± 2 under photoperiod of 16 h.

Table 1. Different concentrations of NAA, IAA BAP, KIN and IBA growth regulators which used in six MS media of Idkawy tomato cultivar for *in vitro* experiment

Medium no.	Different growth regulators
1	MS+ 30 g/L sucrose + 8 g/L agar 8mg/L NAA+0.01mg/L KIN
2	MS+ 30g/L sucrose + 8 g/L agar+ 0.5mg/L IAA
3	MS+ 30 g/L sucrose + 8 g/L agar Without hormone
4	MS+ 30 g/L sucrose + 8 g/L agar+5 mg/L BAP
5	MS+ 30 g/L sucrose + 8 g /L agar 0.1mg/L IBA
6	MS+30 g/L sucrose + 8 g/Lagar+o.2mg/L BAP

NAA=Naphthalene acetic acid, KIN= Kinetin, IAA= Indole acetic acid
BAP= 6- Benzylaminopurine, IBA= Indole butyric acid.

Gamma irradiation treatments

Irradiation was carried out with ^{137}Cs source at the dose rate of 1 Gy/ 2 min 30 sec, at National Centre for Radiation Research and Technology, Cairo, Egypt.

Tomato seeds were soaked in water and exposed to different gamma radiation doses (0, 50, 100, 150, 200 and 250 Gy). The seeds were cultured on solid MS medium **(Murashige and Skoog 1962)** without any hormone. Micropropagation was applied by transferring the nodes onto MS medium supplemented with 8 mg/L NAA and 0.01 mg/L KIN or 0.5 mg/L IAA or 5 mg/L BAP or 0.1 mg/L IBA or o.2 mg/L BAP.

SCoT-PCR molecular markers

Start codon targeted (SCoT) markers for assessment of genetic diversity between the control and irradiated plants were carried out according to **(mansour et al 2018)** .

Extraction of genomic DNA

Total genomic DNA was isolated from the control and irradiated plantlets 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, v/v) were carried out by an additional wash of 97% alcohol (left at -20 °C for one hour), then 70% pre-cooled ethanol, respectively. The yield and quality of DNA were assessed by gel electrophoresis.

Protocol

Ten primers of Start codon targeted (SCoT) were selected from **Collard and Mackill (2009)** as shown in **Table (2)**. Amplification reactions were carried out in a total volume of 25 µl, which contained 250 nM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1 unit Taq polymerase, and 50- 100 ng of template DNA. All reaction volumes were 25 µl which overlaid with a drop of mineral oil. The used thermocycling program 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 for 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 were visualized by staining with ethidium bromide.

Table 2. Primer names, nucleotide sequences and GC% of the ten used SCoT primers.

No.	Primer name	Sequences (5′→3′)	GC %
1	SCoT-1	5'-CAACA**ATG**GCTACCACCA-3'	50
2	SCoT-2	5'-CAACA**ATG**GCTACCACCC-3'	56
3	SCoT-3	5'-CAACA**ATG**GCTACCACCG-3'	56
4	SCoT-4	5'-CAACA**ATG**GCTACCACCT-3'	50
5	SCoT-5	5'-CAACA**ATG**GCTACCACGA-3'	50
6	SCoT-12	5'-ACGAC**ATG**GCGACCAACG-3'	61
7	SCoT-13	5'-ACGAC**ATG**GCGACCATCG-3'	61
8	SCoT-16	5'-ACC**ATG**GCTACCACCGAC-3'	56
9	SCoT-20	5'-ACC**ATG**GCTACCACCGCG-3'	67
10	SCoT-33	5'-CC**ATG**GCTACCACCGCAG-3'	67

Data analysis

The size of SCoT fragments was estimated by comparison with the DNA marker. SCoT fingerprints were recorded in the binary form (1 = presence of a band and 0 = absence) of the amplified products for each sample using GelAnalyzer3 (http://www.gene.ga2h.com), **(Gel Analyzer Version three, 2007).**

RESULTS AND DISCUSSION

***In vitro* tomato plantlets propagation**

Tomato explants were cultured on MS medium with the combination of different growth regulators such as KIN, IAA, 6-BAP or IBA as shown in **Table (3)** to get the best growth rates with any of these hormones. The effect of the used hormones was measured on callus formation and the different plantlet growth rates. The MS medium supplemented with 0.2 mg/L BAP was the best one to be used for tomato explant regeneration.

Effect of gamma radiation on survival of tomato plantlets

The survivals irradiated plantlets were decreased with increasing gamma radiation doses as shown in **Table (4)**. The survival percentages of irradiated tomato plantlets were ranged from 78.75% with 50 Gy to 18.75% with 250 Gy. As well the means of plantlets shoot length (cm) were decreased with increasing gamma radiation doses as shown in **Table (4) and Figure (1)**. They were 2.71 cm with 50 dose Gy and 1.2 cm with 250 Gy dose. The use of ionizing radiation, such as X-rays, gamma rays and neutrons for inducing variation, is well established. Induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanuts, tomato and beans, which are seed propagated. Gamma ray is an ionizing radiation where they react with atoms or molecules to produce free radical in cells. Radicals may have harmful effect or act on rearranging the cell components and this effect may appear on the morphology, physiology, biochemistry and anatomy depending on radiation doses. These effects include changes in 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 **(Kovacs & Keresztes 2002 and Wi et al 2005).** Chronic exposure has largely been used, but does not appear to have any advantages over acute irradiation **(Sigurbjörnsson, 1977).** Generally, gamma irradiation can be used to obtain varieties that are economically important in agriculture, with high productivity and quality **(El-Fiki 1997 and Jain 2010).** They are useful for mutations in breeding programs and *in vitro* mutagenesis in order to develop required features of plants and increase the genetic variability. Many mutant varieties, which are resistant to biotic and abiotic stress and with high quality, have been developed **(Jain et al 2013).** Several attempts of mutagenic treatment on cultured anthers have been reported in higher plants **(Ling et al 1991 and El-Fiki et al 2015).** These results were in accordance with radiation sensitivity test done by **(El-Fiki et al 2015)** for tobacco, (**El-Fiki 1997)** for potato, **(El-Fiki et al 2005)** for alfalfa, **(Norfadzrin et al 2007)** for tomato and okra **(Kiong et al 2008)** for *Orthosiphon stamineus.*

Table 3. The effect of the four growth regulators which added to the six MS media on the growth rates of tomato plants.

Medium no.	Different growth regulators	Plantlets Formation	Callus formation	Shot formation	Root formation
1	MS+8mg/L NAA+0.01mg/L KIN	+	+	+	-
2	MS+0.5mg/L IAA	++	-	+	+
3	MS Without hormone	+++	-	+	+
4	MS+5mg/L BAP	-	-	+	-
5	MS+0.1mg/L IBA	+++	-	+	+
6	MS+o.2 mg/L BAP	++++	-	+	+

Table 4. The effect of gamma radiation doses on number of growing plantlets, bud survival percentage and mean of shoot length from 80 tomato/ plantlets.

Radiation GY/dose	Plant number survival		Mean of shoot length
	No. of growing plantlets	Bud survival percentage	
Control	74	92.5	5.30
50	63	78.75	2.71
100	59	73.75	2.30
150	40	50.00	2.00
200	32	40.00	1.54
250	15	18.75	1.20

SCoT-PCR molecular markers

The polymorphism of irradiated tomato using PCR products

Total genomic DNA from the control and irradiated tomato (Idkawy cultivar) with different gamma radiation doses 50, 100, 150, and 200 Gy were used as templates for SCoT genetic diversity analysis (250 Gy treatment was discarded for its bad results). The ten SCoT primers which selected according to **Collard and Mackill (2009)** as shown in **Table (2)** were used**.** These SCoT primers amplified a total of114 amplicons with a range from 4 with SCoT-4 to 18 with SCoT-5 amplicons per primer with an average of 11.4 amplicons per primer as shown in **Table (5)**. The amplification products were varied from primer to another.

SCoT specific markers

The tested SCoT primers with radiation treatments exhibited that five of these primers were successful to generate specific markers as; SCoT-1, SCoT-2, SCoT-3, SCoT-5 and SCoT-33. The radiatied specific markers were ranged from one fragment with SCoT-1 and SCoT-2 primers, two fragments with SCoT- 5 to five fragments with SCoT-3 and SCoT-33 as shown in **Table (6).** Recently, start codon-targeted (SCoT) markers were successfully used to assess and analyze the plant genetic diversity **(Fang-Yong et al 2014 and Jiang et al 2014 and Zhang et al 2015).**

Table 5. SCoT fragment patterns generated for irradiated tomato with differente gamma radiation doses.

No.	Primer name	MS (bp)	Total no. of fragments	No. of polymorphic fragments	polymorphism %
1	SCoT-1	137 - 686	13	9	69.2
2	ScoT-2	277 - 1166	10	6	60.0
3	SCoT-3	258 - 1302	14	7	50.0
4	SCoT-4	283 - 484	4	2	50.0
5	ScoT-5	161 - 1426	18	12	66.6
6	ScoT-12	172 - 747	15	13	86.6
7	SCoT-13	159 - 1174	12	9	75.0
8	SCoT-16	133 - 684	5	1	20.0
9	ScoT-20	285 - 953	8	3	37.5
10	SCoT-33	255 - 1463	15	7	46.6
Total			114	69	
Average			**11.4**	**6.9**	**56.15**

MS = Molecular size bp= Base paire

SCoT markers have been proved to be useful in genetic diversity studies because of their high reproducibility and great power for the detection of polymorphism **(Cao et al 2006, Sofalian et al 2009, Guo et al 2012 and Hamidi et al 2014).** The SCoT technique is based on the single primer amplified region principle since it uses a single primer as a forward and reverse primer, like the RAPD or ISSR technique. However, PCR amplification using SCoT primers targets gene regions surrounding the ATG initiation codon on both DNA strands. Generally, SCoT markers were reproducible but the factors determining reproducibility as primer length and annealing temperature are not the sole factors **(Collard and Mackill, 2009).** The PCR amplification profile of SCoT markers indicated adominant marker like RAPD and ISSR markers. The SCoT markers are expected to be linked to the functional genes and corresponding traits, thus the amplicons can be converted to gene targeted marker systems **(Xiong et al 2011).** Besides these markers are multilocus, which are helpful in obtaining high genetic polymorphism. The number of amplicons of the SCoT markers observed in the present study is comparable to the results obtained in other studies such as in groundnut **(Xiong et al 2011)**, mango **(Luo et al 2010),** and *Dendrobium nobile* **(Bhattacharyya et al 2013).**

Table 6. SCoT primer names and the specific markers generated for radiation treatments.

Primer name	Specific markers for radiation	
	Negative markers	Positive markers
SCoT-1	219bp	0
SCoT-2	277bp	0
SCoT-3	1276,1026, 559, 476bp	483bp
SCoT-5	259, 231bp	0
SCoT-33	0	1179, 1439, 704, 547, 420bp

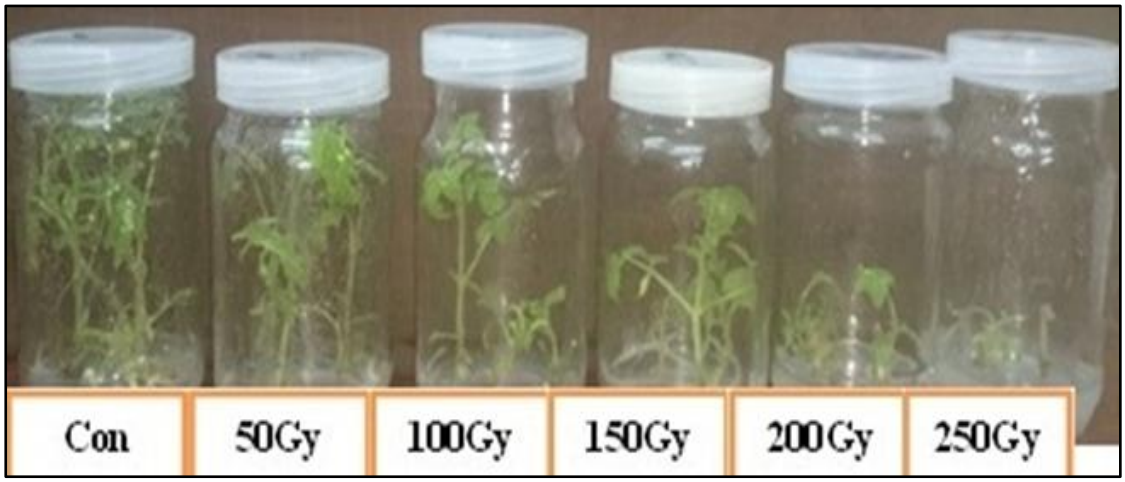

Fig. 1. Effect of gamma radiation on of survival tomato plantlets.

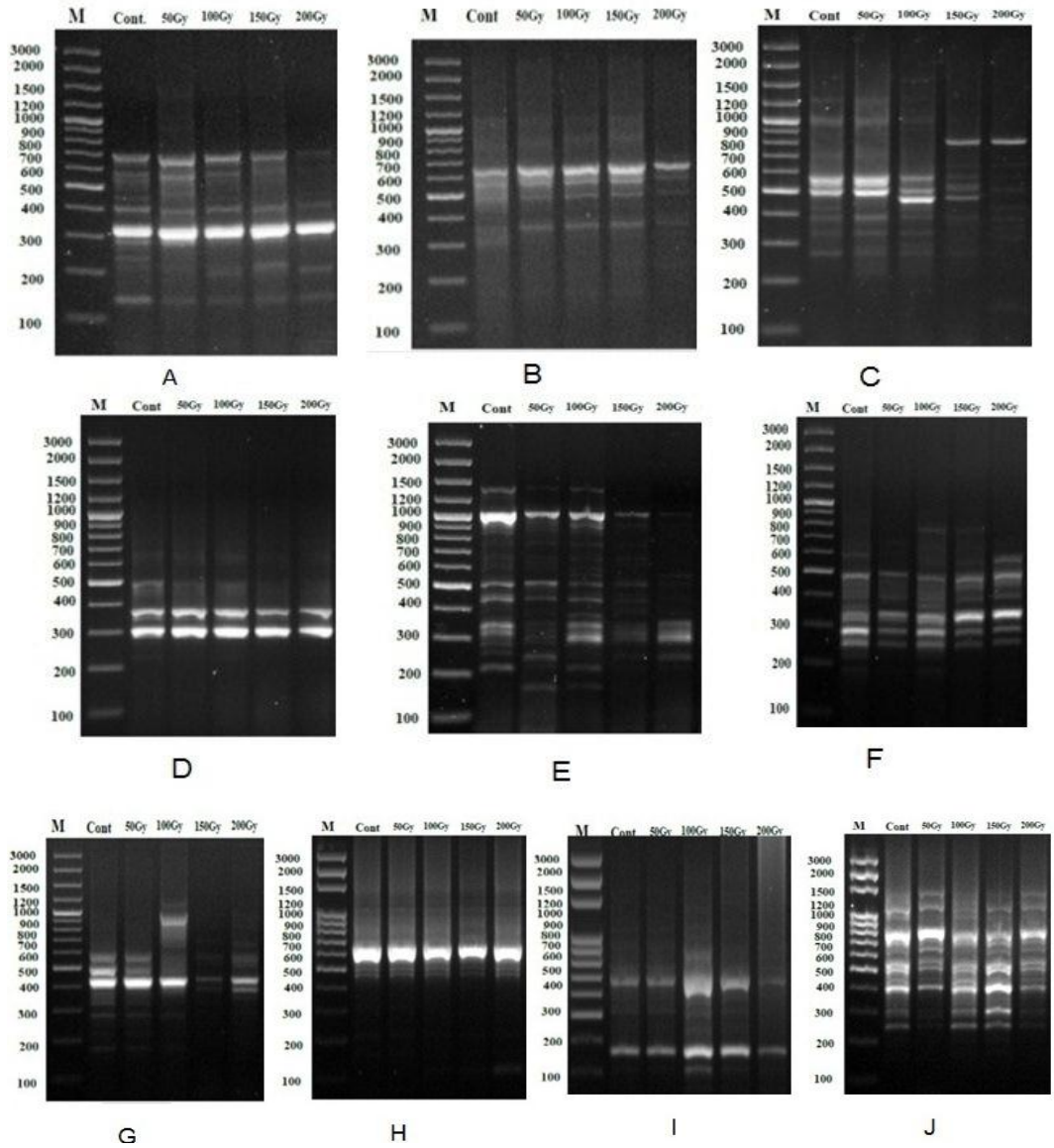

Fig. 2. Representative of SCoT profile for irradiated tomato plants with gamma radiation doses (0, 50,100,150 and 200 Gy) with (A) SCoT 1, (B) SCoT2, (C) SCoT3, (D) SCoT4, (E) SCoT5, (F) SCoT12, (G) SCoT13, (H) SCoT16, (I) SCoT20 and (J) SCoT33 primers

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التنوع الوراثي فى نباتات الطماطم المشععه بإستخدام كشافات إستهداف شفرة البداية Start Codon Targeted (SCoT)

[48]

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تعتبر الطماطم من أهم المحاصيل النباتية العالمية الهامة في مصر. ساهمت تقنيات زراعة الأنسجة في تحسين المحاصيل وذلك عن طريق انتاج الطفرات. قد يكون انتاج الطفرات في المحاصيل الخضرية من خلال زراعة الأنسجة هى الطريقة المثلى لتحسين هذه المحاصيل. تم زراعة الصنف المصري(أدكاوى) معمليا على بيئة موراشيج وسكوج المضاف إليها 0.2 ملجم/ لترمن البنزيل أدينين . أحدثت الجرعات (50 ، 100، 150، 200 و250 جراى) إنخفاضاً ملحوظا فى معدل بقاء ونمو نباتات الطماطم المشعَّعة بزيادة جرعة أشعة جاما. تراوحت نسبة البقاء والحيويه للنباتات المشععة من 75.78٪ بالنسبه للجرعه 50 جراى و 18.75٪ للجرعه 250 جراى، في حين انخفض طول الساق بمعدل 2.71 سم للجرعة 50 جراى و 1.2 سم للجرعة 250 جراى. تم الحصول على 114 حزمة متضاعفة مع استخدام عشر بادئات لتقنية استخدام كشافات استهداف شفرة البداية Start Codon Targeted (SCoT) بمتوسط حزم تتراوح ما بين 4 حزمة مع البادئ SCoT–4 الي 18حزمه مع البادئ SCoT–5. كما أمكن الحصول على 14 حزمة منفردة يمكن استخدامها ككاشفات لمعاملات الإشعاع ، تنقسم الى 6 كاشفات موجبة (ظهرت مع الجرعات الأربعه المستخدمة للإشعاع ولم تظهر بالعينة غير المعاملة كنترول) و8 كاشفات سالبة (إختفت فى جميع العينات المعاملة بالإشعاع بينما كانت موجوده فى العينة غير المعاملة).

الكلمات الدالة: الطماطم، صنف ادكاوي، زراعة الأنسجة، الأشعاع، كاشفات DNA–SCoT متعدد الاشكال

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