

## Induction of Mutations and Genetic Variations in *In Vitro* of Sour Orange Rootstock (*Citrus aurantium*)

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### ABSTRACT

Micronodes were used to propagate Sour orange (*Citrus aurantium*) through In Vitro technique. Also, two cytokinins i.e. Kinetin, 6-benzyl amino purine at concentrations rate of 0.0, 1.0, 2.0, & 3.0 mg/l were employed. The mutagenesis process used chemical mutagens through culturing of in vitro shootlets on MS medium supplemented with different concentrations of Sodium azide (NaN<sub>3</sub>) at rate of 0.04, 0.06, & 0.08%; Colchicine at rate of 0.05, 0.10, & 0.15%. Di methyl sulphate (DMS) at rate of 0.10, 0.30, & 0.50%; and Di ethyl sulphate (DES) at rate of 0.10, 0.30, & 0.50%. Also, physical mutagens were subjected to different doses of UV rays (2, 4, & 6 hours); microwave treatments (200 wat) for 10, 20, & 30 seconds; and Gamma rays 50, 75, & 100 Gray. the highest concentration of BAP (3.0 mg/L) is more effective in increasing Shoot numbers. However, the lowest concentration of chemical mutagens i.e. DMS (0.10%) induced the highest Survival% and Shoot length. while, using Sodium azide (0.04, 0.06, & 0.08%) had a harmful effect on Survival% and Shoot length. On the contrary, using Colchicine improved most parameters under study. However, the Vitrification parameter was noticed significantly with all Sodium azide concentrations i.e. 0.08, 0.06, & 0.04% as well as DES 0.30 & 0.50% concentrations as compared with the other treatments. Furthermore, a Molecular marker (ISSR) was done, by Using eight primers revealing that the ratio of polymorphism 81.6% under physical effect was less than compared with chemical mutagenesis 88.4%.

**KEYWORDS:** : *In Vitro*, Citrus, Chemical Mutation, Physical mutation, ISSR

### 1. INTRODUCTION

Citrus is considered one of the most important fruit crops all over the world, both in terms of planting area and production (Nito 1996;

& Hazarika et al., 2014). In Egypt, citrus fruits are economically important with large scale production. It has great considerable importance in the fruit economy of the country According to

the Ministry of Agriculture statistics, Central Administration of Plant Quarantine (CAPQ), 2021 years. Fruit yields vary considerably with soil type. Nevertheless, good commercial yields are attained on fertile soils.

Sour orange (S.N: *Citrus aurantium*. Family: Rutaceae.) yields much less on the course, of infertile sands, particularly when budded with Valencia orange, and therefore should not be used on such soils (Castle et al., 2016).

A horizontal increase of citrus in newly reclaimed soils is mandatory through the production of new rootstocks capable of tolerating different abiotic factors, such as drought, flooding, salinity, mineral deficiency and toxicity, metal toxicity, heat, cold, soil temperature and oxygen, pH, etc. (Colla et al., 2010; Hartmann et al., 2013; Savvas et al., 2010; Ghrab et al., 2014; & Castle et al., 2016) and different biotic factors such as fungal and bacterial pathogens, virus, diseases, insects or nematodes ( Shokrollah et al., 2009; Roistacher et al., 2010; Louws et al., 2010; & Castle et al., 2016). Employing biotechnology in breeding was helpful in the production of new rootstocks (Annarita, and Laura., 2012). Tissue culture techniques are the most procedures that improve the methods of propagation and produce huge numbers of rootstock plants with complete resemblance to their mother trees (Thorpe., 2007). Pérez-Tornero, and Porrás., (2008) found that embryos from the cultivar 'Eureka' had greater survival, germination percentages, and radical development.

Mutation induction techniques were done either by physically adding radiation i.e Gamma ( Ling et al., 2008; Altaf et al (2009); Xiao et al., 2009; & Sutarto et al., 2009 ), UV (Britt., 1995; Sztatelman et al., 2016; & Dwivedi et al., 2021), and Microwave (Khalafallah, and Sallam., 2009; Jangid et al., 2010; Kesari et al., 2010; Jangid et al., 2010; Cretescu et al., 2013; Soran et al., 2014; & Miler et al., 2018) or by chemical substances i.e Sodium azide ( Raicu and Mixich., 1992; Grant and Salamone., 1994; Al-Qurainy., 2009; AbdulRahaman et al., 2013; & AbdulRahaman et al., 2018 ), Colchicine (Grosser et al., 2014; Eng, W.H. and Ho, W.S., 2019; & Narukulla et al., 2023), Di methyl sulphanate (DMS), and Di ethyl

sulphanate (DES) (Jain., 2005; Ge et al., 2015; & Kaur., 2015 ) which are valuable tools for increasing variability in crop species because spontaneous mutations occur with an extremely low frequency and it is possible to increase variability of economic gamma radiation is probably the most used in fruit trees because of their easy, availability and power of penetration (Moussa., 2006). Moreover, using mutation either with using chemical or physical mutagens are valuable in inducing large scale genetic variabilities which is the main tool of the breeder to raise a new rootstock. Chemical mutagens (Oladosu et al.,2015) Induced mutation delivers desired genetic changes such as early maturation, resistance to various diseases, and semi-dwarfism in plants (Beyaz, and Yildiz., 2017).

The identification of species and varieties, as well as their variations induced by different mutagens, is possible with the aid of various markers like morphological, biochemical, and cytogenetic, but the most efficient are the molecular (DNA) markers, which are based on the differences in their DNA sequence (Bretting, and Widrechner., 1995). In Citrus, markers that use PCR (Polymerase Chain Reaction) technology, have successfully been used, such as RAPD (Asadi, and Isshiki., 2003), SCAR (Nicolosi et al., 2000), and Markers used for this purpose include intersimple sequence repeat (ISSR) primers, extensively useful in establishing the genetic stability of *in vitro*-regenerated plantlets in many crop species (Scarano et al., 2002; Joshi, and Dhawan., 2007; Lakshmanan et al., 2007; Pathak, and Dhawan., 2012; Salis et al., 2017; and Haradzi et al., 2021).

The ultimate goal of this investigation is establishing an easier and more faster protocol using the tissue culture technique as a noble propagation way of citrus rootstocks to alternate to sexual propagation. Besides, an attempt to induce genetic variations in the resulting shootlets, using some physical and chemical mutagens, hopefully, will carry some desirable traits in the future.

## 2. MATERIALS AND METHODS

This study was conducted in the Tissue Culture and Germplasm Conservation Research

Laboratory - Horticulture Research Institute, Agricultural Research Center - Giza, Egypt in cooperation with the Department of Horticulture-Faculty of Agriculture - Benha University during the period from 2021 to 2023.

### 2.1. Shootlets preparation:

Micronodes of Sour orange (*Citrus aurantium*) rootstock were collected from the one-year-old branch and subjected to running water for 5 minutes to get rid of residual fruit tissues then immersed in a soap solution for 5 minutes. After that, those micronodes were immersed in 15% Clorox solution (0.5 NaOCl) commercial bleach with two drops of Tween-20 for 10 minutes then immersed in sterilized distilled water 3 times for 5 minutes each. Then micronodes were cultured on MS medium (Murshig & Skoog) was supplemented with 100 mg/L Myo-inositol, 0.10 mg/l BAP (6-benzylaminopurine), 0.10 mg/l IBA (Indol-3-butyric acid), 30 g/l sucrose, and 7 g/l Difco- Bacto agar as a basal medium. The pH of the medium was adjusted to 5.70 and autoclaved at 121°C for 15 min and the cultured jars were incubated in an incubation room under conditions 16 hours of artificial light (Fluorescent light at 30 mM/m<sup>2</sup>/sec) and 8 hours of dark at an average temperature of 28-30°C. Thus, the following experiments were carried out.

### 2.2. Shootlets proliferation

Two cytokinin types i.e. Kinetin (Kin. 6-furfural amino purine) and 6-benzyl amino purine (BAP) were studied at the rate of 0.0, 1.0, 2.0, and 3.0 mg/L to detect the best cytokinin type and concentration that was able to induce, the highest proliferation of Sour Orange (*Citrus aurantium*).

### 2.3. Mutagenesis process

#### 2.3.1. Chemical mutagenicity effect on morphological characterization

The resultant *in vitro* proliferated shootlets of Sour Orange rootstock were taken and subjected to different mutagenic substances. *In vitro*, shootlets of Sour Orange rootstock were cultured on an MS medium supplemented with four types chemical of mutagens, each at three different concentrations:

1. Sodium azide (NaN<sub>3</sub>) at 0.04%, 0.06%, and 0.08% v/v.
2. Colchicine (C<sub>22</sub> H<sub>25</sub> NO<sub>6</sub>) at 0.05%, 0.10%, and 0.15% w/v.
3. Di methyl sulphanate (DMS) at 0.10%, 0.30%, and 0.50% v/v.
4. Di ethyl sulphanate (DES) at 0.10%, 0.30%, and 0.50% v/v.

The shootlets were cultured on basal MS medium supplemented with DMS or DES at the same concentrations, we found that all explants were vitrified. So, we immersed the explants in a liquid solution of both substances (DMS or EMS) for 30 min, then cultured on MS media. All treatments were kept under the incubation room conditions (16 hours light and 8 hours dark at 28-30°C for 4 weeks). At the end of the incubation period were determined the most effective chemical mutagen with the best concentration which has the ability to induce variable morphological characters (survival percentage, shootlet number, shootlets length, leaves number, FW, and DW), and genetic variation was determined.

#### 2.3.2. Physical mutagenicity effect on morphological characterization

Effects of different physical mutagens on survival and growth parameters of *in vitro* shootlets of Sour orange rootstock were estimated. To study the effects of UV-C, gamma, and Microwave irradiation on the growth of caper shoots, shootlets were placed in small jars containing basal MS medium (without growth regulators), and then subjected to irradiation treatments.

The jars were exposed to UV-type-C rays for three times (2, 4, and 6 h). Employing model G15T8 ultraviolet light UV-C lamp: Philips -TuV-15W- 54 V- 0.34 A, and long at 45 cm, diameter at 2.8 cm, containing 2.0 mg of mercury (Hg), and disinfects water air. UV-C light is a short-wavelength linear tube (254 nm). The distance of exposure was 10 cm from the lamps (Sztatelman et al., 2016; & Dwivedi et al., 2021). The irradiation treatments were done at the Horticulture Research Institute, ARC, Giza, Egypt.

The LG- 42|-MH8265DIS microwave oven with the power of 200 W•cm<sup>-2</sup> and the

frequency of 2.45 GHz was used as the source of radiation for three times (10, 20, and 30 seconds). The irradiation treatments were done at the Horticulture Dep. Moshtohor, Toukh 13736, Egypt.

Exposure to gamma rays with three concentrations (50, 75, and 100 Gray), was performed at the National Centre for Research and Technology in Nasr City, Cairo, Egypt, using  $^{60}\text{Co}$ - $\gamma$  of India gamma cell at dose rate (0.782 Gy/h) (Bahmani et al., 2016; & Babina et al., 2020). to verify the most effective dose that induces the greatest mutations and genetic variabilities without adverse effects on irradiated explants. Irradiation was conducted by using a  $\text{Co60}$  source from a Unit of Gamma Chamber 4000 at the National Center for Radiation Research and Technology Nasr City, Cairo.

The irradiated shootlets were cultured directly after irradiation to a new cultural medium and kept under the incubation room conditions (16 hours light and 8 hours dark at 28-30°C for 4 weeks). At the end of the incubation period morphological characteristics were determined (survival percentage, shootlets number, shootlets length, leaves number, FW, and DW) and genetic variation was determined.

## 2.4. Molecular marker (ISSR-PCR):

### 2.4.1. DNA extraction:

Genomic DNA was extracted from young leaves of mutant shootlets, bulked from 15 different shootlets per genotype following the bio basic protocol. The quantity and quality of genomic DNA was tested by the spectrophotometer and agarose gel electrophoresis.

### 2.4.2. PCR amplification and Electrophoresis

Eight ISSR primers Table(1), were used to amplify the DNA. The ISSR-PCR method was carried out, according to Nagaoka and Ogihara (1997) Amplification reactions were carried out in 26.3 $\mu\text{L}$  volumes, containing (11.0  $\mu\text{L}$  dH<sub>2</sub>O, 3  $\mu\text{L}$  of 10x buffer, 3.0  $\mu\text{L}$  of dNTPs (2.5mM) 4  $\mu\text{L}$  of Mg Cl<sub>2</sub> (25 m M), 3.0  $\mu\text{L}$  primer (2.5  $\mu\text{L}$ ), 0.3  $\mu\text{L}$  of Taq polymerase (5U/  $\mu\text{L}$ ) and 2.0  $\mu\text{L}$  of genomic DNA 50ng/  $\mu\text{L}$ ). Amplification was

performed in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd, Duxford, and Cambridge, U.K). Programmed for an initial denaturation at 94oC for 5 min, 45 cycles of 1 min denaturation at 94oC, 1 min annealing at 40oC, and 2 min extension at 72oC followed by final extension for 10 min at 72oC. The amplified products were separated on 1.5% agarose gel in TBE buffer. The DNA bands were visualized by staining the gels with ethidium bromide and photographed under UV light using gel documentation system.

**Table 1. ISSR primers names and their sequences.**

Primer	Primer name	Sequence
1	HB12	(CAC)3GC
2	HB13	(GAG)3GC
3	TA-1	(AG)10C
4	TA-3	(AGG)6
5	17899B	(CA)6GG
6	UBC-823	(TC)8C
7	UBC-811	(GA)8C
8	UBC-810	(GT)8CA

### 2.4.3. Band scoring and data analysis

The banding pattern of the ISSR markers in the individuals were scored as presence (1) and absence (0) of the band. The pairwise comparisons between the tested genotypes were used to calculate the coefficient of genetic similarity matrix (Gs) according to Nei and Li (1979). The similarity matrix was subjected to cluster analysis using an unweighted pair group method with an arithmetic means (UPGMA) clustering procedure and a dendrogram was generated using the software package MVSP (Multi-Variate Statistical Package).

### 2.5. Data and calculation

shootlets length (cm), Shootlets number, Leaves number, survival percentage, FW (cm), DW(cm), Necrosis, and genetic determined..

### 2.6. Statistical analysis and experimental design

All treatments used in this study were arranged in a Complete Randomized Design (CRD) and replicated 5 times with 3 explants for

each replicate The obtained data were subjected to analysis of variance and statistically analyzed according to Duncan’s multiple range test (Duncan, 1955) at 1% level.

### 3. RESULTS AND DISCUSSION

#### 3.1. Shootlets proliferation

##### 3.1.1. Effect of medium and explant types

Dealing with proliferation, it is clear from Table (2) & Fig (1) that BAP cytokinin is more effective in increasing proliferation than Kin. Also, both concentrations of the two cytokinins 1.0 mg/l BAP, and 1.0 mg/l Kin. were statistically maximized survival percentage parameters in comparison with the other concentrations and cytokinin types. Meanwhile, photo (2) showed Shoot length (6.30 cm) parameter was significantly increased as a lower concentration of BAP 1 mg/l was used about the others. Moreover, lower concentrations of Kin(5.47 cm). took a second rank in increasing shoot length as compared with the higher concentrations of both cytokinin’s BAP & Kin (3.80, and 4.83 cm. respectively). On the other hand, there is a direct

relationship between increasing the concentrations of either BAP or Kin and an increase in the shoot number parameter. The highest BAP concentration (3.0 mg/l) induced the best significant shoot number (7.07 cm) among the others. However, Leaves number increased as low concentrations were used of either BAP or Kin. cytokinin was involved. The highest statistical Leaves number appeared as 1mg /l of Kin (5.90 cm). was used in comparison with the other treatments under study.

Generally, the results conclude that BAP is more effective in increasing proliferation. Also, higher concentrations of either BAP or Kin. induced a better proliferation than lower concentration. However, the reverse was true in the case of Shoot length and Leaves number parameter. These results are going in line with the findings of Muhammad Usman et al., (2005). They found that Shoot proliferation Of Kinnow (*Citrus reticulata*), Sweet lime (*Citrus limmetoides*), and Succari (*Citrus sinensis*) were directly proportion to the increase in the levels of benzyl aminopurine (BA) and naphthalene acetic acid (NAA) in the modified Murashige & Skoog medium.

**Table 2. Effect of two Cytokinin types and concentrations on proliferation of Sour orange.**

Parameters	Survival (%)	Shoot length (cm)	Shoot number (shootlet/explants)	Leaves number (Leaves/shootlet)
<b>Treatments</b>				
<b>Control</b>	62.33 c	5.07 b	2.30 e	3.43 de
<b>BAP 1 mg/l</b>	85.00 a	6.30 a	4.07 cd	4.13 cd
<b>BAP 2 mg/l</b>	74.00 b	3.80 c	4.77 bc	3.17 e
<b>BAP 3 mg/l</b>	65.33 b	2.50 c	7.07 a	3.80 c-e
<b>Kin 1 mg/l</b>	87.33 c	5.47 b	2.13 e	5.90 a
<b>Kin 2 mg/l</b>	83.67 a	4.83 b	3.73 d	5.00 b
<b>Kin 3 mg/l</b>	75.00 b	3.67 c	5.60 b	4.47 bc

Means followed by the same letter within each column are not significantly different from each other at 1% level.

BAP: 6-benzyl amino purine; & Kin: kinetin



**Fig 1. Effect of BAP & Kin on Shootlets of Sour orange.**

### 3.2. Mutagenesis process

#### 3.2.1. Chemical mutagenicity effect on morphological characterization

Data presented in Table (3) and Fig (2) reflected the effect of chemical mutagens on Survival % and growth parameters of *in vitro* Sour orange shootlets. The highest significant Survival percentage (91.33%) occurred in Sour orange when both control and the lowest concentration of DMS (0.30% v/v) were supplemented to the cultured medium about other treatments. While the highest significant shoot length CHC 0.05% (1.93 cm). However, using of Sodium azide with different concentrations (0.04%, 0.06%, & 0.08% v/v) induced a significant harmful effect on Survival percentages (5.00, 5.33, and 2.00% ) and Shoot length parameters(0.77, 0.47, 0.27 cm). On contrary, Leaves number, Fresh weight, and Dry weight as well as Necrosis parameters increased significantly by using 0.05% v/v Colchicine (4.33, 2.70 g, 0.39 g, and Necrosis 5. respectively). as compared with most other treatments. On the other hand, Vitrification parameters were statistically increased by using 0.04% v/v Sodium azide (94.67%) and DES at 0.30% (100%), 0.50% (100%), and CHC.0.05% (87.33) & 0.10% (82.27%) treatments.

#### 3.2.2. Chemical mutagenicity effect on genetic variabilities parameters of Sour orange shootlets

Using 8 ISSR primers on Sour orange shootlets were subjected to different chemical mutagens (Control, CHC 0.05%, CHC 0.10%, CHC 0.15%, DMS 0.10%, DMS 0.30%, DMS 0.50%. DES 0.10%, DES 0.30%, and DES 0.50% v/v) showed 69 variables bands were of them 37 bands as polymorphic (without Unique) with a ratio of 53.6%, while found 24 unique bands with a ratio of 34.8% and these bands ranged in size between 515 to 183bp with mean of polymorphism 88.4%. An asset of eight ISSR primers revealed 69 variable bands of them 61 polymorphic and 8 bands as monomorphic with a ratio of 11.6% among ten orange genotypes. A 100% polymorphism was scored for two primers (P1 and P3). While a low polymorphism of 66.67% was scored for primer 2.

Meanwhile, the other three primers (P4, P5, and P8) produced polymorphism at 87.5% but primer 6 gave 91% while, primer 7 scored 75 polymorphism. The total percentage of polymorphic markers for eight primers in the examined 10 accessions is 88.4%, which indicates a high level of genetic variation among Sour orange plantlets that were subjected to different chemical mutagens.

**Table 3. Chemical mutagenicity effect on morphological characterization.**

Parameters Treatments	Survival %	Shoot L. (cm)	Shoot No.	Leaves No.	Vitrification	FW (g)	DW (g)	Necrosis
<b>SA 0.04%</b>	5.00 f	0.77 c-e	1.33 ab	2.67 ab	94.67 a	0.66 d	0.10 b-d	1.66 c
<b>SA 0.06%</b>	5.33 f	0.47 de	1.00 b	2.67 ab	82.67 a-c	0.51 d	0.07 cd	1.33 c
<b>SA 0.08%</b>	2.00 f	0.27 e	1.00 b	2.00 b	96.67 a-c	0.32 d	0.04 d	1.00 c
<b>DMS 0.1%</b>	72.33 b	1.03 b-d	1.33 ab	3.33 ab	58.33 b-d	1.03 cd	0.12 b-d	4.67 ab
<b>DMS 0.3%</b>	91.33 a	1.33 a-c	1.67 ab	3.00 ab	65.00 cd	1.73 bc	0.23 a-d	4.00 ab
<b>DMS 0.5%</b>	62.67 c	0.90 b-e	1.33 ab	2.33 b	41.33 e	1.83 a-c	0.29 ab	3.33 b
<b>DES 0.1%</b>	66.00 bc	1.43 ab	1.67 ab	2.33 b	40.00 e	2.23	0.25 a-c	5.00 a
<b>DES 0.3%</b>	67.00 bc	0.63 c-e	1.00 b	2.00 b	100.00 a	0.83 d	0.09 b-d	3.67 ab
<b>DES 0.5%</b>	43.00 d	0.47 de	1.00 b	1.67 b	100.00 a	0.63 d	0.03 d	3.33 b
<b>CHC 0.05%</b>	67.67 bc	1.93 a	2.33 a	4.33 a	87.33 ab	2.70 a	0.39 a	5.00 a
<b>CHC 0.10%</b>	49.00 d	1.27 a-c	1.33 ab	2.67 ab	82.67 a-c	1.10 cd	0.18 a-d	4.67 ab
<b>CHC 0.15%</b>	31.67 e	0.90 b-e	1.00 b	2.67 ab	67.33 b-d	0.84 d	0.03 d	4.33 ab

Means followed by the same letter within each column are not significantly different from each other at 1% level.  
SA: Sodium azide; DMS: Di methyl sulphamate; DES: Di ethyl sulphamate; & CHC: Colchicine (C22 H25 NO6 )



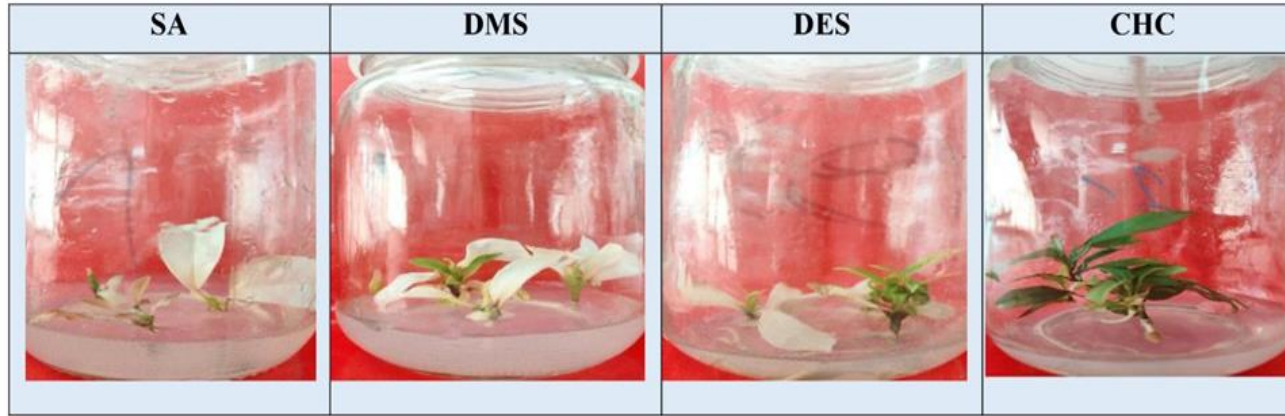


Fig 2. Chemical mutagenicity effect on morphological characterization.

Table 4. Gel profile for ten Sour orange Shootlets using eight ISSR primers

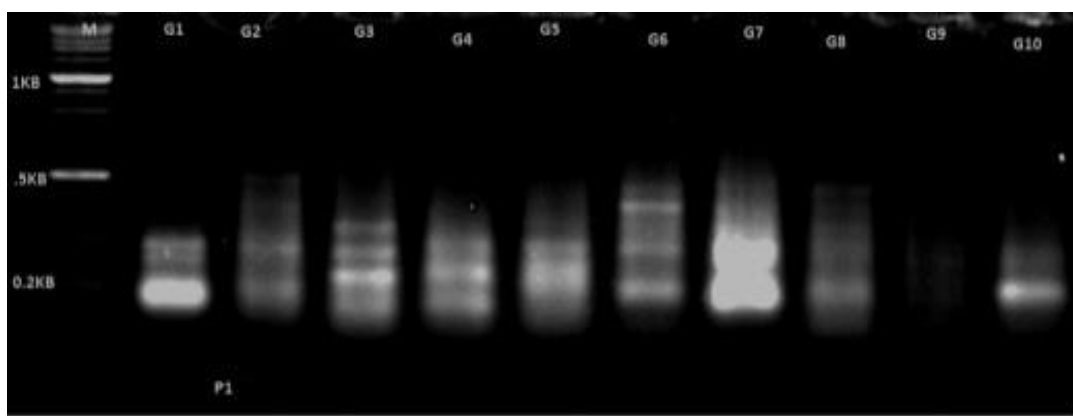
Primers code	Primers name	Total number of bands	Polymorphic (without Unique)	Unique bands	Monomorphic bands	Polymorphic (with Unique)	Polymorphism (%)	Mean of band frequency	Range size of bands (bp)
P1	HB12	11	5	6	0	11	100%	0.236	456:188
P2	HB13	6	4	0	2	4	66.667 %	0.583	475:210
P3	TA-1	9	4	5	0	9	100%	0.3	468:218
P4	TA-3	8	5	2	1	7	87.5%	0.463	515:194
P5	17899B	8	6	1	1	7	87.5%	0.463	493:199
P6	UBC-823	11	6	4	1	10	91%	0.282	500:183
P7	UBC-811	8	4	2	2	6	75%	0.463	468:187
P8	UBC-810	8	3	4	1	7	87.5%	0.362	493:336
<b>Total</b>	-	69 (100%)	37 (53.6%)	24 (34.8%)	8(11.6%)	61(88.4%)	88.4%	0.394	515:183

Using 8 ISSR primers detected 34 positive markers (Table 4) These unique bands scoured by seven primers were the high number 6 by P1 and

the low number 1 by primer P5 as single positive markers.

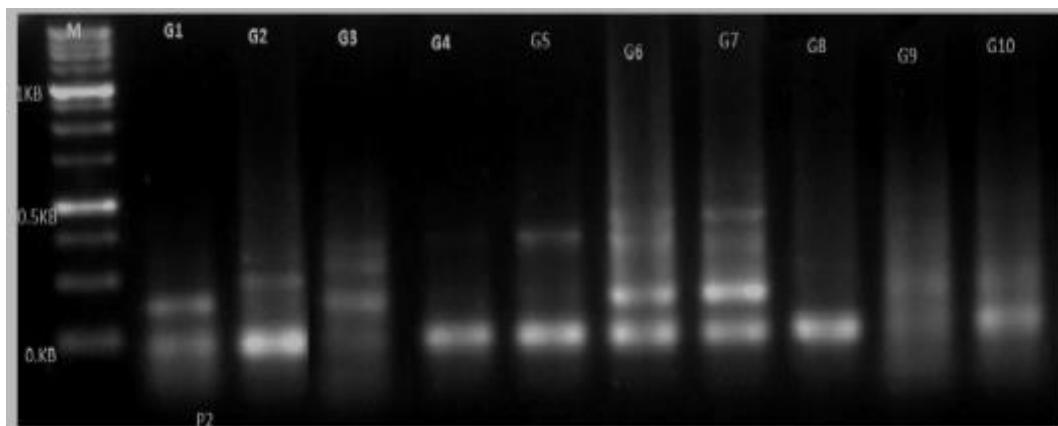
**Table 5. Genetic markers under chemicals mutagen using eight ISSR primers.**

Primers	MS	Genotypes No.									
		Cont.	CHC 0.05%	CHC 0.1%	CHC 0.15%	DMS 0.1%	DMS 0.3%	DMS 0.5%	DES 0.1%	DES 0.3%	DES 0.5%
P1(6)	378	0	0	0	0	0	1	0	0	0	0
	376	0	0	0	0	0	0	0	0	0	1
	225	0	0	0	0	0	0	0	0	0	1
	203	0	0	0	0	1	0	0	0	0	0
	189	0	0	0	0	0	1	0	0	0	0
	188	0	0	0	0	0	0	1	0	0	0
P3(5)	469	0	0	0	0	0	0	1	0	0	0
	384	0	0	0	0	0	0	1	0	0	0
	344	0	0	1	0	0	0	0	0	0	0
	295	0	0	0	0	0	0	1	0	0	0
	219	0	0	0	0	0	0	1	0	0	0
P4(2)	337	0	0	1	0	0	0	0	0	0	0
	298	0	0	0	0	0	0	0	1	0	0
P5(1)	250	0	0	0	0	0	0	0	0	1	0
	367	0	0	1	0	0	0	0	0	0	0
P6(4)	240	0	0	1	0	0	0	0	0	0	0
	217	0	0	0	0	0	0	0	0	1	0
	210	0	0	1	0	0	0	0	0	0	0
P7(2)	340	0	0	1	0	0	0	0	0	0	0
	256	1	0	0	0	0	0	0	0	0	0
	253	0	0	0	0	0	0	1	0	0	0
P8(4)	246	0	1	0	0	0	0	0	0	0	0
	207	0	1	0	0	0	0	0	0	0	0
	136	1	0	0	0	0	0	0	0	0	0

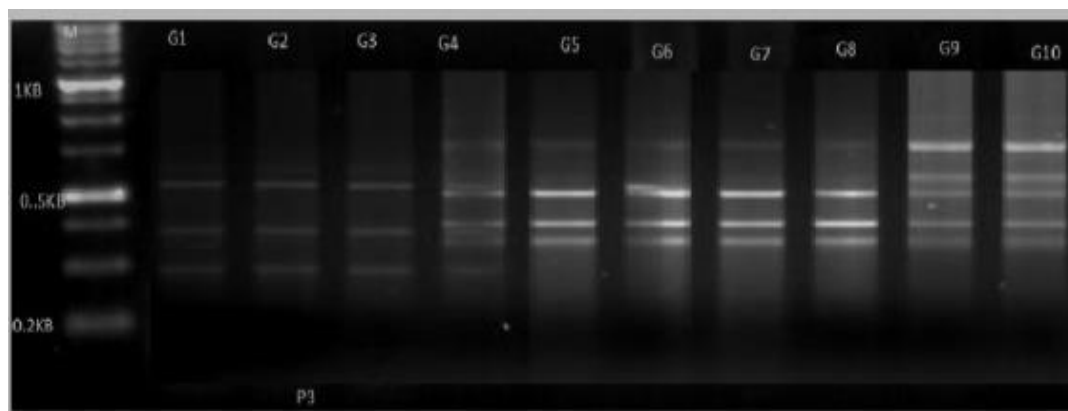


**Fig 3. ISSR-PCR of *C.aurantium* , Control, CHC 0.05%, CHC 0.10%, CHC 0.15%, DMS 0.10%, DMS 0.30%, DMS 0.50%. DES 0.10%, DES 0.30%, and DES 0.50% via HB12 primer.**

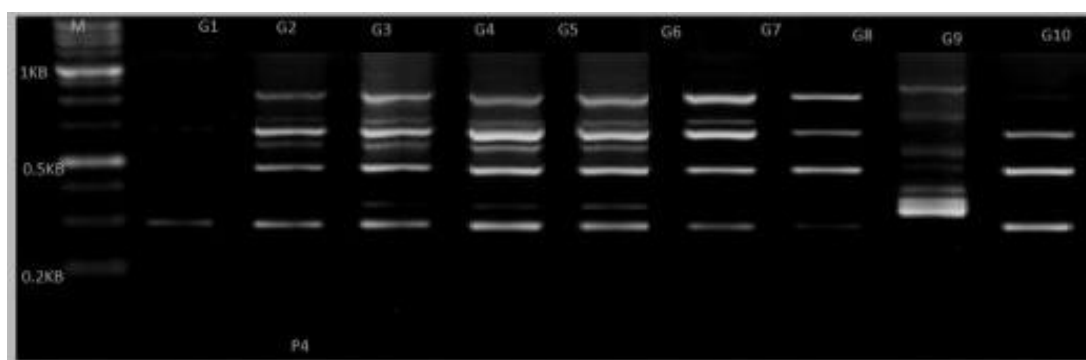




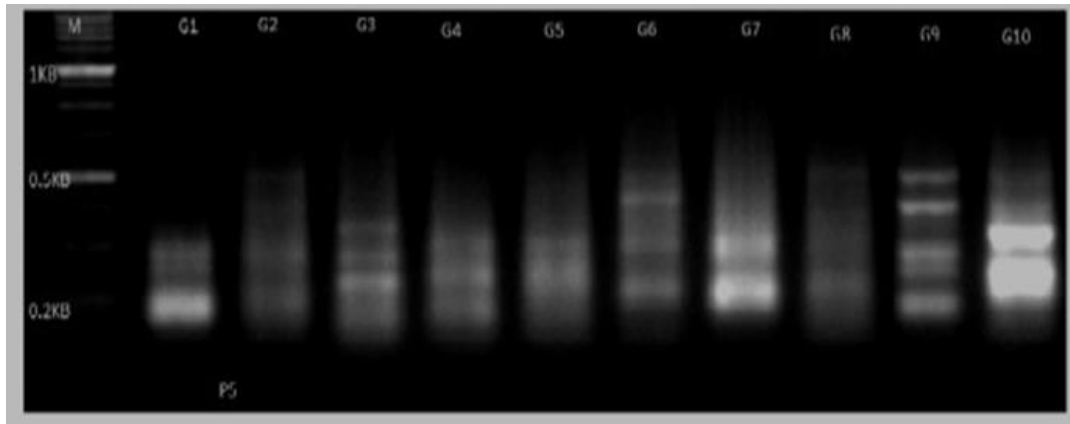
**Fig 4. ISSR-PCR of *C.aurantium* , Control, CHC 0.05%, CHC 0.10%, CHC 0.15%, DMS 0.10%, DMS 0.30%, DMS 0.50%. DES 0.10%, DES 0.30%, and DES 0.50% via HB13 primer.**



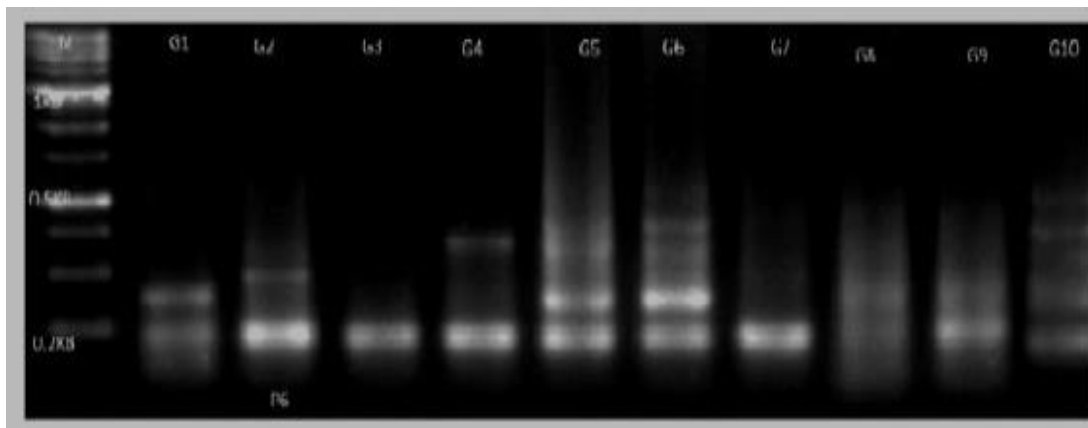
**Fig 5. ISSR-PCR of *C.aurantium* , Control, CHC 0.05%, CHC 0.10%, CHC 0.15%, DMS 0.10%, DMS 0.30%, DMS 0.50%. DES 0.10%, DES 0.30%, and DES 0.50% via TA-1 primer.**



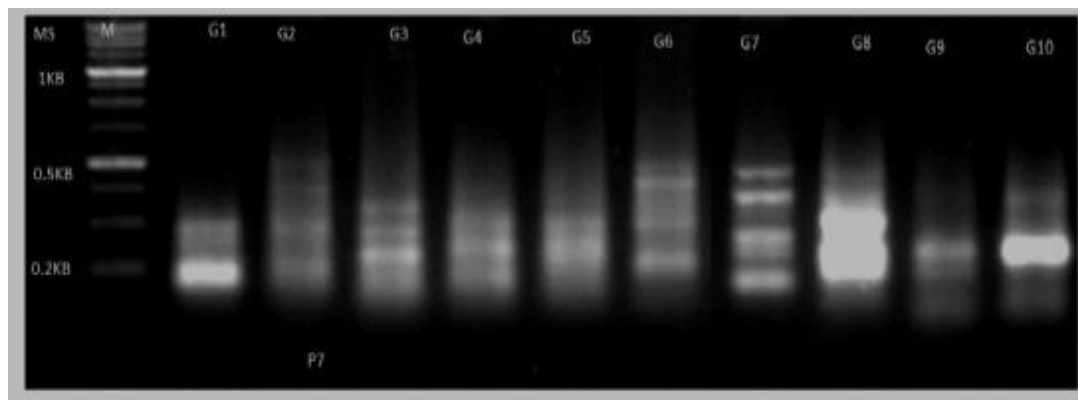
**Fig 6. ISSR-PCR of *C.aurantium*, Control, CHC 0.05%, CHC 0.10%, CHC 0.15%, DMS 0.10%, DMS 0.30%, DMS 0.50%. DES 0.10%, DES 0.30%, and DES 0.50% via TA-3 primer.**



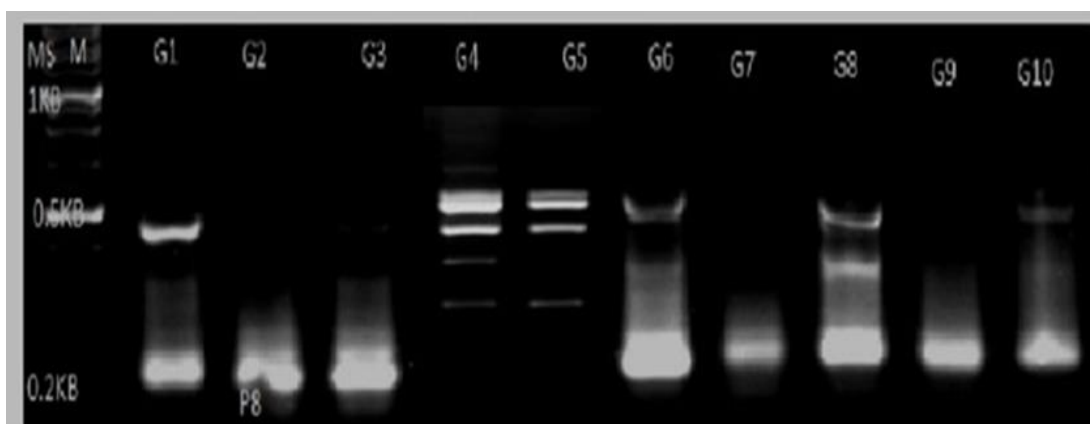
**Fig 7. ISSR-PCR of *C.aurantium* , Control, CHC 0.05%, CHC 0.10%, CHC 0.15%, DMS 0.10%, DMS 0.30%, DMS 0.50%. DES 0.10%, DES 0.30%, and DES 0.50% via 17899B primer.**



**Fig 8. ISSR-PCR of *C.aurantium* , Control, CHC 0.05%, CHC 0.10%, CHC 0.15%, DMS 0.10%, DMS 0.30%, DMS 0.50%. DES 0.10%, DES 0.30%, and DES 0.50% via UBC-823 primer.**



**Fig 9. ISSR-PCR of *C.aurantium* , Control, CHC 0.05%, CHC 0.10%, CHC 0.15%, DMS 0.10%, DMS 0.30%, DMS 0.50%. DES 0.10%, DES 0.30%, and DES 0.50% via UBC-811 primer.**



**Fig 10. ISSR-PCR of *C.aurantium* , Control, CHC 0.05%, CHC 0.10%, CHC 0.15%, DMS 0.10%, DMS 0.30%, DMS 0.50%. DES 0.10%, DES 0.30%, and DES 0.50% via UBC-810 primer.**

**3.2.3. Physical mutagenicity effect on morphological characterization:**

Table (6) & Fig (11) showed the effect of physical mutagens on Survival and growth parameters of *in vitro* Sour orange shootlets. Subjecting *in vitro* shootlets to different physical mutagens at low doses of irradiation either by UV 2, Mic1, Gamma 1, and Gamma 3 rays (85, 85, 100, and 85 % . respectively) statistically improved

the survival % as compared to the higher doses of different physical mutagens. However, the Shoot length parameter was significantly increased as the shootlets were subjected to different doses of UV 6, Mic 1 & 2, and Gamma 1, 2, & 3 ( 2.1, 1.73, 1.77, 2.40, 1.93, and 2.20 cm. respectively) about others. On the other hand, significance disappeared among treatments when Shoot number, fresh weight, dry weight, and Necrosis parameters were considered.

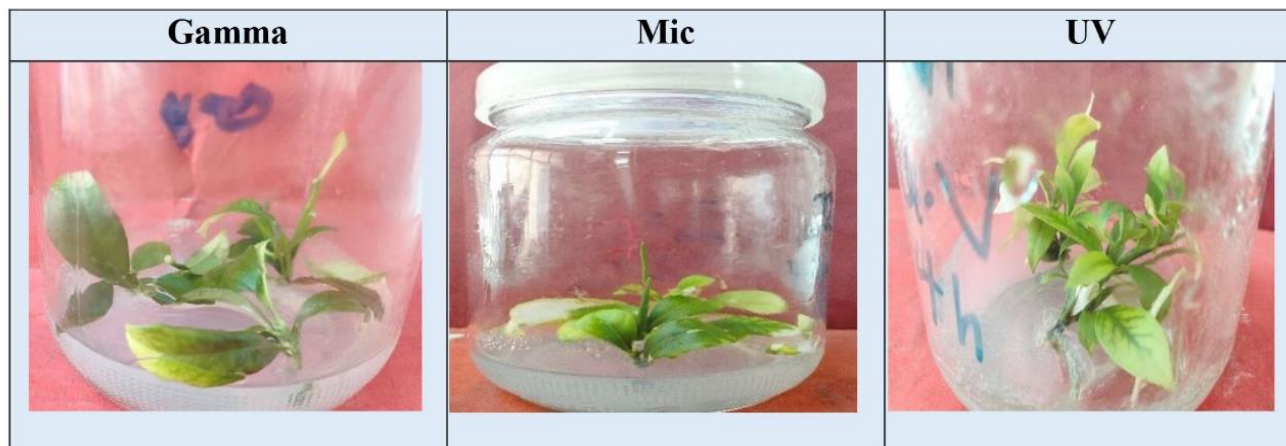
**Table 6. Physical mutagenicity effect on morphological characterization.**

Parameters Treatments	Survival %	Shoot L. (cm)	Shoot No.	Leaves No.	FW (cm)	DW (cm)	Necrosis
<b>UV 1</b>	85 ab	2.1 ab	1.33 a	4.33 ab	1.08 a	0.10 a	4.67 a
<b>UV 2</b>	69 c	1.33 ab	1.00 a	3.55 b	0.89 a	0.10 a	4.33 a
<b>UV 3</b>	78 bc	2.1 ab	2.17 a	3.00 b	1.03 a	0.097 a	5.00 a
<b>Mic 1</b>	85 ab	1.73 ab	1.78 a	3.66 ab	2.01 a	0.29 a	4.33 a
<b>Mic 2</b>	77.33 bc	1.77 ab	1.83 a	6.17 ab	1.90 a	0.25 a	4.33 a
<b>Mic3</b>	85 ab	1.10 b	1.00 a	3.60 ab	1.93 a	0.17 a	4.00 a
<b>Gamma 1</b>	100 a	2.40 a	1.33 a	3.33 b	1.31 a	0.10 a	5.00 a
<b>Gamma 2</b>	83.33 bc	1.93 ab	1.00 a	7.67 a	2.11 a	0.28 a	4.33 a
<b>Gamma 3</b>	85 ab	2.20 a	1.10 a	6.33 ab	2.29 a	0.15 a	5.00 a

Means followed by the same letter within each column are not significantly different from each other at 1% level.

UV: ultraviolet rays; Mic: Microwave; & Gamma: Gamma rays

( UV1: 2 hours; UV2: 4 hours; UV3: 6 hours; Mic1:10 seconds; Mic2: 20 seconds; Mic3: 30 seconds; Gamma1: 50 Gray; Gamma2: 75 Gray; & Gamma3: 100 Gray )



**Fig 11. Effect of different physical mutagens on Survival and growth parameters of Sour orange shootlets.**

#### **3.2.4. Physical mutagenicity effect on genetic variability's parameters of Sour orange shootlets**

Using 8 ISSR primers on Sour orange shootlets were subjected to different doses of physical mutagens (Control, UV2h, UV4h, Gamma100Gy, and Mic1) showed 64 variables bands were of them 41 bands as polymorphic (without Unique) with a ratio of 64.1%, while found 11 unique bands with a ratio of 17.2% and these bands ranged in size between 515 to 183bp with mean of polymorphism 81.3%. An asset of eight ISSR primers revealed 64 variable bands of them 52 polymorphic with a ratio of 81.3% and 12

bands as monomorphic with a ratio of 18.8% among five sour orange genotypes.

A high polymorphism of 90% was scored for primer 7, While a low polymorphism of 71.4% was scored for primer 5. Meanwhile, primer 2 produced polymorphism at 87.5%, primer 6 scored 83.3% polymorphism, primer 3 scored 81.8% polymorphism, primer 8 scored 80% polymorphism, primer 1 scored 77.8% polymorphism, and primer 4 scored 75% polymorphism. The total percentage of polymorphic markers for eight primers in the examined 5 accessions is 81.3%, which indicates a high level of genetic variation among Sour orange shootlets that were subjected to different doses of physical mutagens.

**Table 7. Gel profile using eight ISSR primers; P1: P8 of five Sour orange Shootlets under physical mutagenesis.**

Primers code	Primers name	Total number of bands	Polymorphic (without Unique)	Unique bands	Monomorphic bands	Polymorphic (with Unique)	Polymorphism (%)	Range size of bands (bp)
<b>P1</b>	HB12	9	5	2	2	7	77.8%	456:188
<b>P2</b>	HB13	8	6	1	1	7	87.5%	475:210
<b>P3</b>	TA-1	11	7	2	2	9	81.8%	468:218
<b>P4</b>	TA-3	8	5	1	2	6	75%	515:194
<b>P5</b>	17899B	7	4	1	2	5	71.4%	493:199
<b>P6</b>	UBC-823	6	5	0	1	5	83.3%	500:183
<b>P7</b>	UBC-811	10	6	3	1	9	90%	468:187
<b>P8</b>	UBC-810	5	3	1	1	4	80%	493:336
<b>Total</b>	-	64(100%)	41(64.1%)	11(17.2%)	12(18.8%)	52(81.2%)	81.3%	515:183

**Table 8.. Genetic markers under physical mutagens of five Sour orange Shootlets.**

Primers	MS(bp)	Genotypes No.					Band type
		Cont.	UV2h	UV4h	Gamma 100gy	Mic1	
<b>P1(2)</b>	640	1	1	1	1	0	Negative marker
	315	0	0	0	0	1	Positive marker
<b>P2(1)</b>	435	0	1	1	1	1	Negative marker
	560	1	1	1	1	0	Negative marker
<b>P3(2)</b>	412	0	0	0	0	1	Positive marker
	270	0	1	1	1	1	Negative marker
<b>P5(1)</b>	410	0	0	0	0	1	Positive marker
	815	1	1	1	0	1	Negative marker
<b>P7(3)</b>	430	0	0	0	1	0	Positive marker
	310	1	1	1	0	1	Negative marker
<b>P8(1)</b>	580	1	0	0	0	0	Positive marker
<b>Total(11)</b>	-	2	4	4	4	5	N-(6)&P+(5)

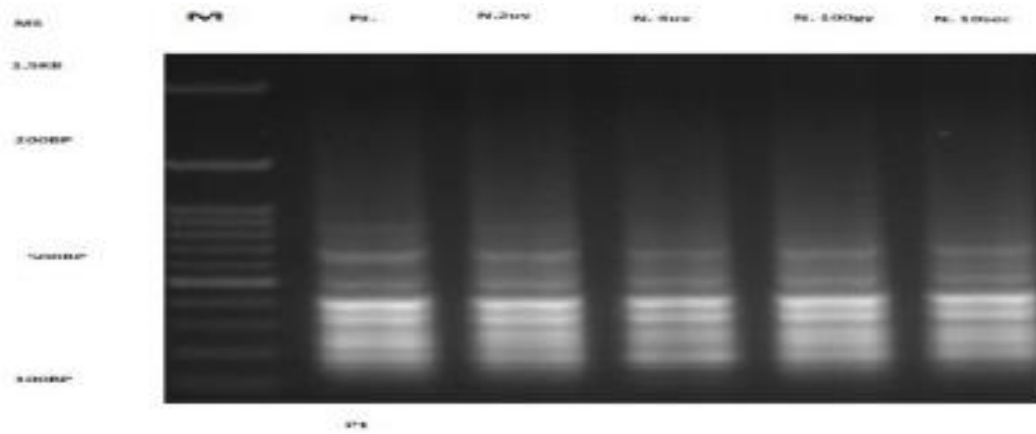


Fig 12. ISSR-PCR of *C.aurantium* , Control, UV2h, UV4h, Gamma100Gy, and Mic1 via HB12 primer.

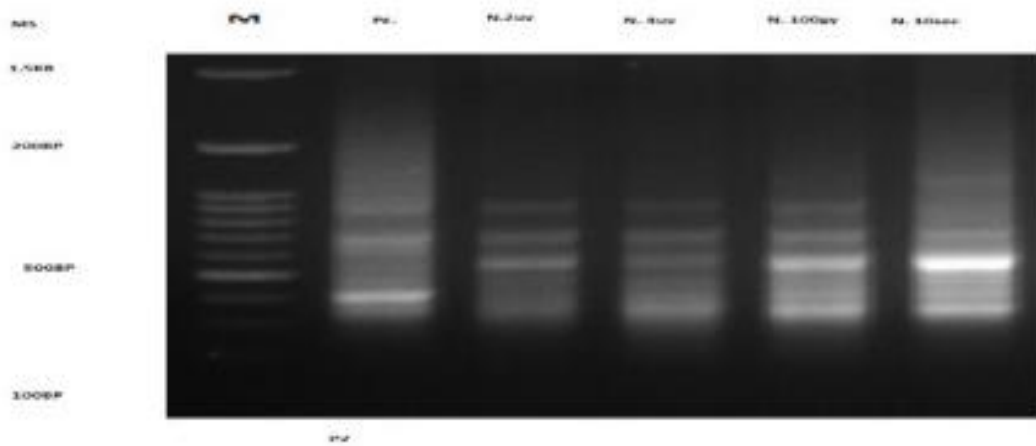


Fig 13. ISSR-PCR of *C.aurantium* , Control, UV2h, UV4h, Gamma100Gy, and Mic1 via HB13 primer

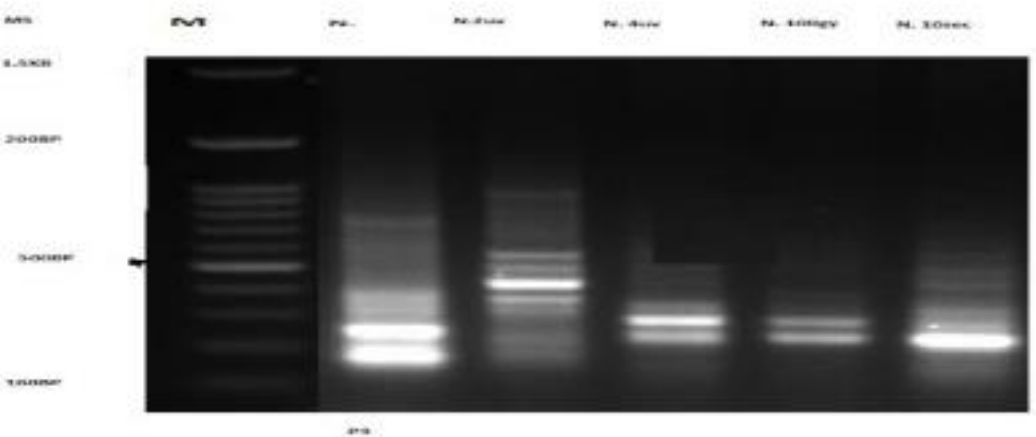


Fig 14. ISSR-PCR of *C.aurantium* , Control, UV2h, UV4h, Gamma100Gy, and Mic1 via TA-1 primer

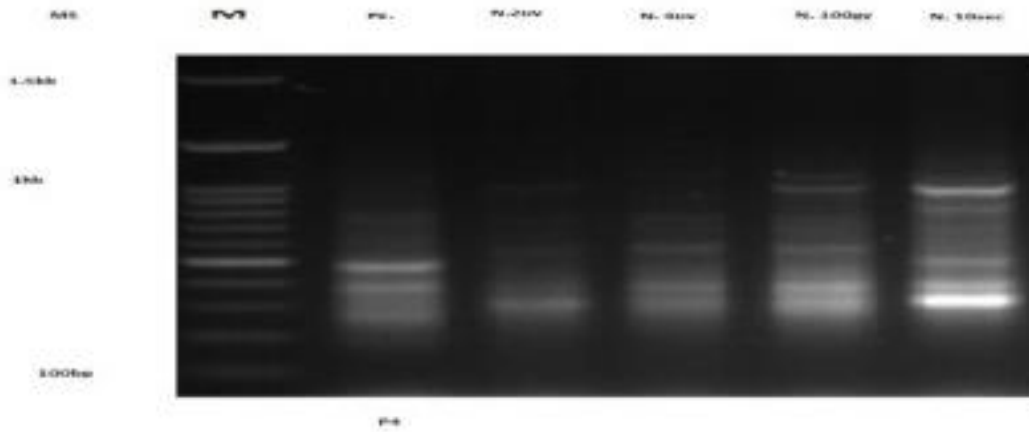


Fig 15. ISSR-PCR of *C.aurantium* , Control, UV2h, UV4h, Gamma100Gy, and Mic1 via TA-3 primer

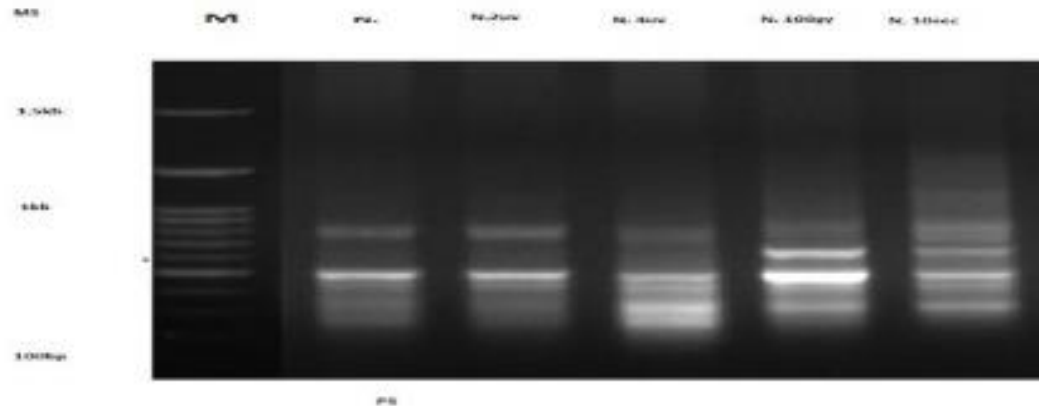


Fig 16. ISSR-PCR of *C.aurantium* , Control, UV2h, UV4h, Gamma100Gy, and Mic1 via 17899B primer

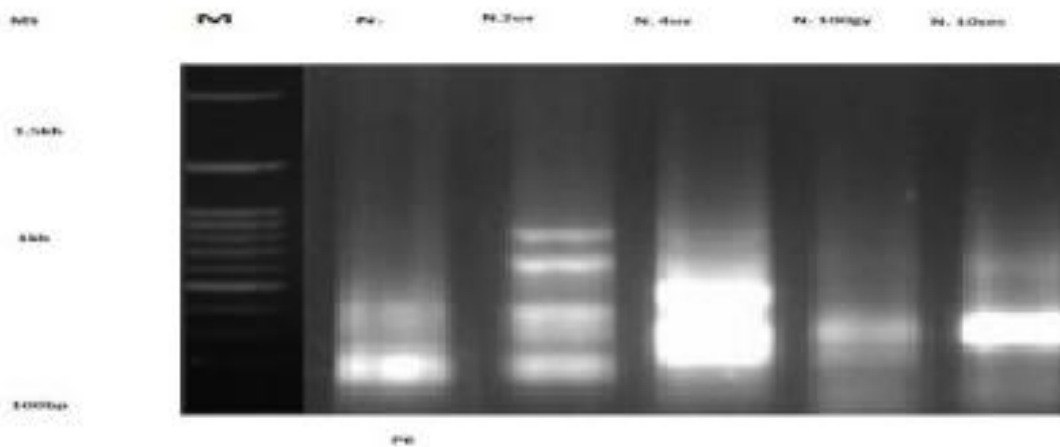
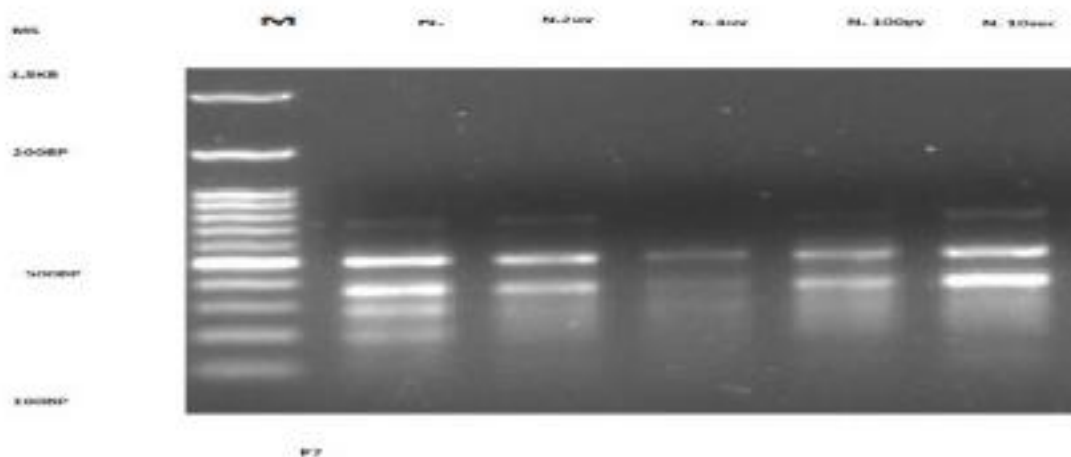
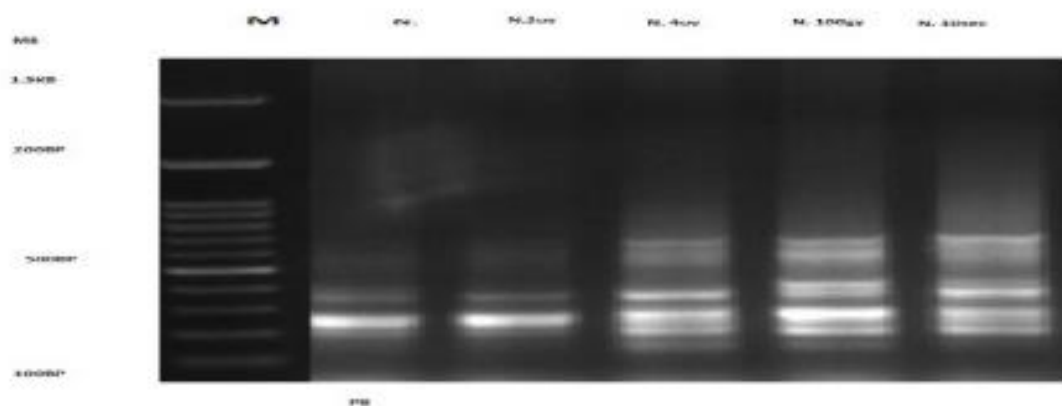


Fig 17. ISSR-PCR of *C.aurantium* , Control, UV2h, UV4h, Gamma100Gy, and Mic1 via UBC-823 primer





**Fig 18. ISSR-PCR of *C.aurantium* , Control, UV2h, UV4h, Gamma100Gy, and Mic1 via UBC-811 primer**



**Fig 19. ISSR-PCR of *C.aurantium* , Control, UV2h, UV4h, Gamma100Gy, and Mic1 via UBC-810 primer**

#### 4. CONCLUSION

This study showed that BAP cytokinin is more effective in increasing proliferation than Kin. The highest BAP concentration (3 mg/l) induced the best Shoot number among the others. the highest statistical leaves number appeared as (1mg/l) of Kin was used in comparison with the other treatments under study.

It has appeared that using the lowest concentration of DMS (0.10% v/v) induced the highest Survival percentage and Shoot length parameters about other treatments. However, using Sodium azaid with different concentrations (0.04%, 0.06%, & 0.08% v/v) had a harmful effect on Survival percentages and Shoot length parameters. On the contrary, Shoot number,

Leaves number, Fresh weight, and Dry weight as well as Necrosis parameters were increased by using 0.50% v/v Colchicine as compared with most other treatments. On the other hand, the Vertification parameter was increased by using of 0.04% v/v Sodium azaid as well as DES at 0.3%, 0.5% v/v, and Colchicine at 0.05% & 0.10% v/v treatments. Using of 0.10% Colchicine improved most parameters under study i.e. Survival %, Shoot length, Leaves number, Dry weight, and Necrosis. However, the Vitrification parameter was noticed significantly with all Sodium azide concentrations i.e. 0.08%, 0.06%, and 0.04% v/v as well as DES 0.3% & 0.5% v/v concentrations as compared with the others. On the other hand, Shoot number parameter showed no significance

among chemical mutagens with different concentrations involved in the study.

It was revealed using eight primers from ISSRs with different Effects of chemical mutagens on genetic variability. The ten genotypes revealed high scour of bands by primer1 and primer 6 showed eleven variable bands while primer2 revealed a little scour of bands where revealed six bands.

Subjecting *in vitro* shootlets to different physical mutagens at low doses of irradiation either by UV2, Mic1, or Gamma 1 rays was statistically improved the survival% as compared to the higher doses of different physical mutagens. It is noticed that the Shoot length parameter was increased as the shootlets were subjected to different doses of UV, Mic 1 & 2, and Gamma 1, 2, & 3 among others. On the other hand, significance disappeared among treatments when Shoot number, fresh weight, dry weight, and Necrosis parameters were considered.

Using eight ISSR primers revealed 49 polymorphic bands with a ratio of polymorphism 81.6 for five Sour orange shootlets under physical effect were less than comparing with chemical mutagenesis 88.4

## 5. REFERENCES

- AbdulRahaman AA, Afolabi AA, Zhigila DA, Oladele FA and Al Sahli AA (2018).** Morpho-anatomical effects of sodium azide and nitrous acid on *Citrullus lanatus* (Thunb.) Matsum. & Nakai (*Cucurbitaceae*) and *Moringa oleifera* Lam.(*Moringaceae*). *Hoehnea*, 45, 225-237.
- AbdulRahaman AA, Afolabi AA, Olayinka BU, Mustapha OT, Abdulkareem KA and Oladele FA (2013).** Effects of sodium azide and nitrous acid on the morphology and leaf Anatomy of *Jatropha curcas* L. (*Euphorbiaceae*). *International Journal of Phytofuel and Allied Sciences* 2: 30-41.
- Al-Qurainy F (2009).** Effects of sodium azide on growth and yield traits of *Eruca sativa* L. *World Applied Sciences Journal* 7: 220-226.
- Altaf N, Khan AR, Ali L and Bhatti IA (2009).** In vitro culture of kinnow explants. *Pakistan Journal of Botany*, 41(2), 597-602.
- Annarita L and Laura MRR (2012).** Recent advance in plant in vitro culture. Page 8.. Edited by Annarita Leva and Laura M. R. Rinaldi.
- Asadi AA and Isshiki S (2003).** Molecular characterization and genetic diversity among Japanese acid Citrus (*Citrus* spp.) based on RAPD markers. *Journal of Horticultural Science and Biotechnology* 78(1)108-112.
- Beyaz R and Yildiz M (2017).** The use of gamma irradiation in plant mutation breeding. *Plant Engineering* 33-46.
- Bretting PK and Widrechner MP (1995).** Genetic markers and plant genetic resource management. In: Janick J, Wiley J (Eds). *Plant Breeding Reviews* pp 11-86.
- Britt AB (1995).** Repair of DNA damage induced by ultraviolet radiation. *Plant physiology*, 108(3), 891.
- Castle WS, Bowman KD, Grosser JW, Futch SH and Graham JH (2016).** Florida Citrus rootstock selection guide (3rd Ed). UF/IFAS Extension Service, University of Florida.
- Colla G, Rouphael Y, Leonardi C and Bie Z (2010).** Role of grafting in vegetable crops grown under saline conditions. *Scientia Horticulturae* 127(2):147-155.
- Cretescu I, Rodica C, Velicevici G, Ropciuc S and Buzamat G (2013).** Response of barley seedlings to microwaves at 2.45 GHz. *Anim. Sci. Biotechnol.* 46 (1), 185-191.
- Duncan HB (1955).** Multiple range and multiple F.test-Biometrics, 11:- 42.1.
- Eng WH and Ho WS (2019).** Polyploidization using colchicine in horticultural plants: A review. *Sci. Hortic.*, 246, 604-617.
- Ge H, Li Y, Fu H, Long G, Luo L, Li R and Deng Z (2015).** Production of sweet orange somaclones tolerant to citrus canker disease by in vitro mutagenesis with EMS. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 123, 29-38.
- Ghrab M, Mimoun MB, Masmoudi MM and Mechlia NB (2014).** Chilling trends in a

warm production area and their impact on flowering and fruiting of peach trees. *Scientia Horticulturae* 178:87-94.

- Grant WF and Salamone MF (1994).** Comparative mutagenicity of chemicals selected for test in the international program on chemical safety collaborative study on plant systems for the detection of environmental mutagens. *Mutation Research* 310: 187-209.
- Grosser JW, Kainth D and Dutt M (2014).** Production of colchicine-induced autotetraploids in pummelo (*Citrus grandis* Osbeck) through indirect organogenesis. *HortScience*, 49, 944–948.
- Gruszka D, Szarejko I and Maluszynski M (2012).** Sodium Azide as Mutagen. In: *Plant Mutation Breeding and Biotechnology*. CABI International, Willingford, UK., pp. 159-166.
- Haradzi NA, Khor SP, Subramaniam S and Chew BL (2021).** Regeneration and micropropagation of Meyer lemon (*Citrus x meyeri*) supported by polymorphism analysis via molecular markers. *Scientia Horticulturae*, 286, 110225.
- Hartmann HT, Kester DE, Davies FT and Geneve RL (2013).** *Hartmann and Kester's plant propagation: principles and practices*. 8th Edn. Pearson Education Limited, Harlow.
- Hazarika TK, Hazarika BN and Shukla AC (2014).** Genetic variability and phylogenetic relationships studies of genus *Citrus* L. with the application of molecular markers. *Genet. Resour. Crop Evol.* 61(8),1441–1454
- Jain SM (2005).** *In vitro* mutagenesis for improving date palm (*Phoenix dactylifera* L.). *Emirates Journal of Food and Agriculture*. 24:400-407.
- Jangid RK, Sharma R, Sudarsan Y, Eapen S, Singh G and Purohit AK (2010).** Microwave treatment induced mutations and altered gene expression in *Vigna aconitifolia*. *Biologia Plantarum*, 54, 703-706.
- Joshi P and Dhawan V (2007).** Assessment of genetic fidelity of micro propagated *Swertia chirayita* plantlets by ISSR marker assay. *Biologia Plantarum* 51: 22-26.
- Kaur S (2015).** Effect of mutagens on regeneration and growth of in vitro grown epicotyl segments of rough lemon seedlings (*Citrus jambhiri* Lush.). *Journal of Applied and Natural Science*, 7(1), 459-465.
- Kesari KK, Behari J and Kumar S (2010).** Mutagenic response of 2.45 GHz radiation exposure on rat brain. *Int. J. Radiat. Biol.* 86 (4). 334–343.
- Khalafallah AA and Sallam MS (2009).** Response of maize seedlings to microwaves at 945 MHz. *Rom. J. Biophys.* 19 (1), 49–62.
- Lakshmanan V, Venkataramareddy SR and Neelwarne B (2007).** Molecular analysis of genetic stability in long-term micro propagated shoots of banana using RAPD and ISSR markers. *Electron Journal Biotechnology* 10: 1-8.
- Ling APK, Chia JY, Hussein S and Harun AR (2008).** Physiological responses of *Citrus sinensis* to gamma irradiation. *World Applied Sciences Journal*, 5(1), 12-19.
- Louws FJ, Rivard CL and Kubota C (2010).** Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods, and weeds. *Scientia Horticulturae* 127:127-146.
- Miler N and Kulus D (2018).** Microwave treatment can induce chrysanthemum phenotypic and genetic changes. *Scientia Horticulturae*, 227, 223-233.
- Murashige T and Skoog F (1962).** A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15: 473- 497.
- Nagaoka T and Ogihara Y (1997).** Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theoretical and applied genetics*, 94, 597-602.
- Narukulla V, Lahane Y, Fiske K, Pandey S and Ziogas V (2023).** Induction of Polyploidy in *Citrus* Rootstocks through In Vitro

- Colchicine Treatment of Seed-Derived Explants. *Agronomy*, 13(6), 1442.
- Nei M and Li WH (1979)**. Mathematical model for studying genetic variations in terms of restriction endonucleases. *Proc. Nat. Acad. Sci.*, 76: 5269-5273.
- Nicolosi E, Deng ZN, Gentile A, La Malfa S, Continella G and Tribulato E (2000)**. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. *Theoretical and Applied Genetics* 100:1155-1166.
- Nito N (1996)**. In: Second International Crop Science Congress. Nov. 17 – 25, New Delhi, India. Page 40 (Abs).
- Oladosu Y, Rafii MY, Absullah N, Hussain G, Ramli A, Rahim HA, Miah G, Pathak H and Dhawan V (2012)**. ISSR assay for ascertaining genetic fidelity of micropropagated plants of apple rootstock Merton 793. *In Vitro Cell. Dev. Biol. - Plant* 48, 137–143.
- Pérez-Tornero O and Porras I (2008)**. Assessment of polyembryony in lemon: Rescue and in vitro culture of immature embryos. *Plant Cell Tissue Organ Cult.*, 93, 173–180.
- Raicu P and Mixich F (1992)**. Cytogenetic effects of sodium azide encapsulated in liposomes on heteroploides cell cultures. *Mutation Research* 283: 215-219.
- Roistacher CN, Graca JV and Muller GW (2010)**. Cross protection against Citrus tristeza virus- a review. *Proceedings of the 17th Conference of the International Organization of Citrus Virologists (OCV' 10)*, Adana, Turkey pp 1-27.
- Salis C, Papadakis IE, Kintzios S and Hagidimitriou M (2017)**. *In vitro* propagation and assessment of genetic relationships of citrus rootstocks using ISSR molecular markers. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 45(2), 383-391.
- Savvas D, Colla G, Roupael Y, Schwarz D (2010)**. Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. *Scientia Horticulturae* 127:156-161.
- Scarano MT, Abbate L, Ferrante S, Lucretti S and Tusa N (2002)**. ISSR-PCR technique: a useful method for characterizing new allotetraploid somatic hybrids of mandarin. *Plant Cell Reports* 20:1162-1166.
- Shokrollah H, Abdullah TL, Sijam K, Abdullah SNA and Abdullah NAP (2009)**. Differential reaction of Citrus species in Malaysia to huanglongbing (HLB) disease using grafting method. *American Journal of Agricultural and Biological Sciences* 4(1):32-38.
- Soran ML, Stan M, Niinemets Ü and Copolovici L (2014)**. Influence of microwave frequency electromagnetic radiation on terpene emission and content in aromatic plants. *Journal of plant physiology*, 171(15), 1436-1443.
- Sutarto I, Agisimanto D and Supriyanto A (2009)**. Development of promising seedless Citrus mutants through gamma irradiation. In *Induced Plant Mutations in the Genomics Era*, edited by Shu, Q.Y. Rome: FAO of the United Nations.
- Sztatelman O, Labuz J, Hermanowicz P, Banaś AK, Zglobicki P, Aggarwal C and Gabryś H (2016)**. Fine-tuning chloroplast movements through physical interactions between phototropins. *Journal of Experimental Botany*, 67(17), 4963-4978.
- Thorpe T (2007)**. History of plant tissue culture. *Molecular Biotechnology* 37:169 – 180.
- Usman M (2015)**. Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnol. Biotec. Eq.* 30, 1–16.
- Xiao JP, Chen LG, Xie M, Liu HL and Ye WQ (2009)**. Identification of AFLP fragments linked to seedlessness in Ponkan mandarin (*Citrus reticulata* Blanco) and conversion to SCAR markers. *Scientia Horticulturae* 121(4): 505-510

## الملخص العربي

### استحداث طفرات وتباينات جينية في المعمل لأصل النارنج

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أجريت هذه الدراسة خلال الفترة من ٢٠٢٣-٢٠٢١ بمعمل زراعة الأنسجة وحفظ الأصول الوراثية التابع لمعهد بحوث البساتين بمركز البحوث الزراعية بالحيزة وبالتعاون مع قسم البساتين بكلية الزراعة بمشتهر -جامعة بنها بهدف استحداث طفرات و تباينات وراثيه في أصل النارنج وذلك لإنتاج أصول جديده تكون أكثر تقوقا عن الأصول القديمة والتي تكون حساسه لكثير من المشاكل التي تواجه الموالح وكذا دراسة تأثير محدثات الطفرة (الفيزيقيه والكيمائيه) لإنتاج طفرات وتباينات وراثية وتأثيرها على السلوك الوراثي وكذا دراسة وعمل بصمة وراثية لهذه النباتات. حيث أخذت العقد الصغيرة واستخدمت كأجزاء نباتيه من اصل النارنج وزراعتها علي بيئة موراشيغ وسكوج ثم عمل زيادة عددية للنباتات الناتجة وأوضحت النتائج تفوق واضح للزيادة العددية للبنزائل أمينو بيورين بمقارنتها بالكينيتين كما تفوقت التركيزات المنخفضه (١,٠ و ٢,٠ ملجم /لتر) من البنزائل أمينو بيورين والكينيتين في تحسين نسبة البقاء وقياسات طول النمو وعدد الأوراق بمقارنتها بالتركيزات الأخرى بينما وجد أن التركيزات العاليه (٣,٠ ملجم/لتر) من ال ٦-بنزائل أمينو بيورين ساعدت على أفضل زياده عدديه بمقارنتها بالتركيزات المنخفضه ووجد أن الزياده العدديه يمكن زيادتها بطريقه كبيره عند استخدام تركيز عالي من ال ٦-بنزائل أمينو بيورين كذلك وجد أن استخدام التركيزات أو الجرعات المنخفضه من محدثات الطفره الكيمائيه أو الفيزيقيه تحت التجربه أعطت أفضل نسبة بقاء وقياسات النمو داخل الأنابيب بينما أعطت التركيزات أو الجرعات العاليه منها على أكبر تغيرات واختلافات وراثيه كما أظهرته البصمه الوراثيه. وباستخدام ثمانية بادئات من ISSRs تم الكشف عن نسبة تعدد الأشكال ٨١,٦% لخمس نباتات نارنج (البرتقال الحامض) تحت التأثير الفيزيائي حيث كانت أقل من مقارنتها مع الطفرات الكيمائية ٨٨,٤%.