Molecular Genetic Studies on (*Ficus Palmata & Ficus Carica Subsp Rupestris.*) Under The Effect of Gamma Radiation by Tissue Culture Techniques.

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ABSTRACT

Ficus spp are used as medicine and are used to reducing the risk of cancer and heart disease. The phytochemical screening of the *Ficus palmata* plant extracts showed the presence of alkaloids, tannins, flavonoids, terpenoids and cardiac glycosides. This investigation was undertaken by using tissue culture technique on *Ficus palmata & Ficus carica subsp rupestris*. Study the effect of gamma rays doses of 0.0, 20, 30 and 40 Gy on callus production and the secondary plant constituents. The highest callus fresh weightand callus induction were achieved by callus derived from (leaves & stem) explants, which were cultured on (MS) medium supplemented with different concentrations of 2,4-D at 2.0 (mg/l) and Kin at 0.2 (mg/l). Polymerase chain reaction (PCR) amplification using eight ISSR primers showed polymorphic patterns with different response to gamma radiation doses. found genetic differences at the molecular level using ISSR with gamma doses compared to control. The total number of polymorphic bands was 239 bands (99.58% polymorphism). Found that there was genetic stability between the mother plants and product tissue culture for plants (*Ficus palmata* & *Ficus carica subsp rupestris*). The chemical composition of *Ficus palmata* was studied by means of GC-MS analysis under the effect of gamma radiation. The best doses of gamma were 30 & 40 Gy, as they increased the secondary compounds of *Ficus palmata*.

Keywords: *Ficus palmata & Ficus carica subsp rupestris*, gamma radiation, callus induction, ISSR-PCR, GC-MS.

INTRODUCTION

Sinai is one of the most important areas in Egypt. It has many natural plants. The plants contain nutritional value suitable for grazing and have medicinal uses. Plants which are grown in Sinai resistance drought and some stresses. Figs (*Ficus carica subsp rupestris & Ficus palmata* Forssk) belong to the family Moraceae. *Ficus carica subsp rupestris* is a very rare plant and *Ficus palmata* Forssk is the wild rootstock of cultivated figs. It is a rare plant (Bolous, 2009). *Ficus palmata* Forssk is used in making various products such as squash, jam and jelly. It contains a very juicy fruit. The fruits contain chiefly sugars and mucilage and are principally used as an item of diet in several cases of constipation and in diseases of the lungs and the bladder. The phytochemical screening of the *Ficus palmata* plant extracts showed the presence of alkaloids, tannins, flavonoids, terpenoids and cardiac glycosides. Fruit extracts were analyzed against cervical cancer cell lines for antiproliferative activity, while aqueous extract of *Ficus palmata* leaves showed dose-dependent anti-carcinogenic action. *Ficus palmata* total plant extract was found to show hepatoprotective, nephroprotective, antiulcer and anticoagulant activity. (Yogesh *et al*., 2014). Figs are used as medicine and are also used to reduce the risk of cancer and heart disease. It is rich in polyphenolic compounds, anthocyanin and flavonoids, mainly Kaempferol. It shows potent antioxidant potential. (Sharma *et al*., 2017). Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells. γ- rays have been reported to affect differentially the morphology, anatomy, biochemistry, and physiology of plants depending on the irradiation level. This effect include change in plant cellular structure and metabolism, e.g. dilution of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidant system, and accumulation of phenolic compounds (Kovacs and Keresztes, 2002, Kim *et al*., 2004 and Wi *et al*., 2005). Inter simple sequence repeats (ISSRs) (Zietkiewicz *et al*. 1994). These markers have advantages and disadvantages but produce reliable information on genetic diversity in genetically neutral regions Karp (2002). Inter simple sequence repeat (ISSR)-PCR is a technique which involves the use of microsatellite sequences as primers in a polymerase chain reaction to generate multilocus markers. It is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) with

the universality of (RAPD) random amplified polymorphic DNA (Reddy *et al*., 2002). Medicinal plants have been used by all civilizations as a source of medicines since ancient times. The study intends to evaluate the gas chromatography–mass spectrometry (GC/MS) analysis of the chemical constituents of callus leaves of *Ficus palmata*. This study will be carried out by Hossam *et al.,* (2019) and aims to find out if the plant contains a number of compounds that could have been used in ancient times for medicinal purposes. This study aimed the molecular behaviors of *Ficus palmata & Ficus carica subsp rupestris* under effect of Gamma Radiation by tissue culture techniques.

MATERIALS AND METHODS

This study was carried out by cooperation between genetic lab Botany Department Faculty of Agriculture Al-Azhar University and the unit of Biotechnology, Genetic Resources Department, Desert Research Center, Cairo, Egypt.

Collection of Plant material

Explants of (*Ficus palmata* & *Ficus carica subsp rupestris*) were obtained from the city of Saint Catherine, South Sinai Governorate.

Gamma irradiation source:

Co was used as a source of gamma rays with a dose rate of 1 Krad /1hour 3 sec. Irradiation Callus with four doses of gamma rays at the National Center for Radiation Research and Technology, Naser City, Cairo, Egypt.

Irradiation by gamma

(*Ficus palmata* & *Ficus carica subsp rupestris*) callus was irradiated with four doses of gamma rays (0.0, 20, 30 and 40 Gy).

Tissue culture experiment.

Initiation of callus from leaves and stem.

This experiment was planned to study the effect of explant type (Stem and Leaves) and the different concentrations of auxin (2,4-D) and cytokinins (Kin) on callus formation. Therefore, uniform explants (0.1 cm) from the different old *in vitro plantlets* (Stem and Leaves) derived from *Ficus palmata* & *Ficus carica subsp rupestris* were cultured in sterile jars containing 30 ml of (MS) basal medium + 3.0% sucrose + 0.7% agar. (Murashige and Skoog 1962).

Callus induction

Excised leaf discs (1×1cm2) of 30 day old stem *in vitro* plants were used for callus induction using the basal MS medium (Table 1) supplemented with a combination of different concentrations of auxin (2,4-D) and cytokinins (kin) (0.0, 2 mg/l 2,4-D + 0.2 Kin and 4 mg/l 2,4-D + 0.4 Kin) (Hemeid *et al*., 2010). All the cultures were transferred to a growth room for 4 weeks. All the cultures were incubated in the culture room under controlled conditions, where the temperature was maintained at 25±2ºC. Cultures were incubated in darkness until a callus was formed, then transferred to a light intensity of about 3000 lux. The photo-period under light conditions was l6h light and 8h dark.

DNA isolation

The bulk DNA extraction was performed using the DNeasy Mini Kit (QIAGEN). PCR was performed in 30-µl volume tubes according to Williams *et al.* (1990) and Yang and Quiros (1993).

Polymerase chain reaction (PCR) condition for ISSR.

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 37º C, and 2 min at 72º C. the reaction was finally stored at 72º C for 10 min (Atawodi *et al*., 2010).

Statistical Analysis ISSR

ISSR bands were manually scored as present (1) or absent (0) for estimating the similarity among all tested samples. Pairwise comparisons were calculated using Jaccard's coefficient. The similarity values found were used to generate a consensus tree using the Unweighted Pair Group Method Analysis (UPGMA) using Gene Tools-gel analysis software of SPSS (ver. 21). Polymorphism percentage was estimated by dividing the number of polymorphic bands over the total number of bands.

GC-MS Analysis.

The chemical composition of cullus Leaves of *Ficus palmata* was studied by means of GC-MS analysis under effect of Gamma Radiation. Hossam *et al.,* (2019). Extract prepared by using one gram of fresh weight callus was extracted with acetyl acetate. This analysis was performed to study gamma ray on secondary compounds for (*Ficus palmata*) (Ivanov *et al*., 2018)

Methods:

GC-MS analysis were carried in The Regional Center for Mycology and Biotechnology – AL-Azhar University. Separation of metabolites was performed on DB-5 column (30 m x 0.25 mm) at temperature program: 40°C (5min) to 275°C (5min) at5°C/min 5. The injector temperature was 300°C. Helium was used as the carrier gas at a flow about 1.0 ml/min pulsed splitless. The mass spectrometric detector was operated in an electron impact ionization mode with an ionizing energy of 70 eV. (Abubakar *et al*., 2016).

RESULTS AND DISCUSSION

Callus induction:

Excised leaf discs (1×1cm2) of 30 day old stem *in vitro* plants were used for callus induction using the basal MS medium supplemented with a combination of different concentrations of auxin (2,4-D) and cytokinins (kin) (0.0, 2 mg/l 2,4-D + 0.2 Kin and 4 mg/l 2,4-D + 0.4 Kin). All the cultures were transferred to a growth room for 4 weeks (Hemaid *et al*., 2010). All the cultures were incubated in the culture room under controlled conditions, where the temperature was maintained at 25±2ºC. Cultures were incubated in darkness until a callus was formed, then transferred to light intensity of about 3000 lux. The photo-period under light conditions was l6h light and 8h dark. The data in table 3 and figure 1 indicated that the highest survival percentage (100%) was obtained on MS medium supplemented with 2 mg/l 2,4-D + 0.2 Kin, whereas the lowest one was found with control (68%). The highest callus formation percentage (91%) was obtained on MS medium supplemented with 2 mg/l 2,4-D + 0.2 Kin (Table 3 and figure 1). The best result for formation and multiplication of explants were cultured on MS medium supplemented with different concentrations of 2 mg/l 2,4-D + 0.2 mg/l Kin. These findings have been confirmed by others (Hemaid *et al*., 2010). MS supplemented with 2.0 mg/l 2,4-D and 0.2 mg/l kinetin was shown to be the optimal medium for callus development. The combination of 2,4-D and kinetin in varied concentrations in callus induction for leaf segments exhibited varying effects on callus induction and weight in all treatments. When all of the treatments were compared, it was discovered that 2,4-D is required for callus induction and that adding kinetin at a certain concentration increases callus growth in leaf culture. This whole outcome showed that 2,4-D was essential for callus induction and kinetin was useful to promoting callus growth (Joyner *et al*., 2010 and Dalila *et al*., 2013).

Trying to produce new genotypes using mutagens

Callus induction from the leaf & stem segment & shoot tip for plants (*Ficus palmata* & *Ficus carica subsp rupestris)* After that, the irradiation callus of (*Ficus palmata* & *Ficus carica subsp rupestris*) was subjected to three doses of gamma rays (cobalt 60) (0.0, 20, 30, and 40 Gy). The best doses are 30 & 40 Gy. The tested doses of gamma irradiation did not cause callus mortality (Fig 2). These results were confirmed by (Ferreira *et al*., 2009) studying *in vitro* induced mutations in plants (*Ficus carica* L) by gamma radiation (10, 20, 30, 40 and 50) Gy. Doses greater than 30 Gy prevent the formation of roots. Doses up to 50 Gy do not cause plant death. It was the best dose when using 30 Gy.

Molecular genetic marker by ISSR:

Genetic of differences were studied at the molecular level using ISSR molecular analysis of treated callus by gamma rays. The effect was studied by eight primers of ISSR showed a difference between the control for plants (*Ficus palmata* & *Ficus carica subsp rupestris*) and their mutants. The amplification results of the *Ficus palmata* & *Ficus carica subsp* rupestris obtained by the studied primers are described in table 4 and figure 3. The following are the amplification results of the *Ficus palmata* & *Ficus carica subsp rupestris* obtained by the analyzed primers. Eight ISSR primers (ISSR1, ISSR3, ISSR5, ISSR7, ISSR11, ISSR12, ISSR13 and ISSR14) produced 240 bands within the DNA template representing callus (Control), 20, 30 and 40 Gy callus treated with different doses of gamma radiation of *Ficus palmata* & *Ficus carica subsp rupestris.* The Primer ISSR1 revealed 35 bands divided into 1 monomorphic and 18 unique bands with a percentage of polymorphism (97.143%). While the primer ISSR3 primer revealed 43 bands divided into 29 polymorphic and 14 unique bands with a percentage of polymorphism (100%). The primer ISSR5 primer revealed 24 bands divided into 14 polymorphic and 10 unique bands with a percentage of polymorphism (100%). On the other hand, the primer ISSR7 primer revealed 53 bands divided into 31 polymorphic and 22 unique bands with a percentage of polymorphism (100%). As opposed to the primer, ISSR11 primer revealed 24 bands divided into 15 polymorphic and 9 unique bands with a

percentage of polymorphism (100%). In contrast to the primer ISSR12 primer revealed 17 bands divided into 10 polymorphic and 7 unique bands with a percentage of polymorphism (100%). On the other side the primer ISSR13 primer revealed 24 bands divided into 15 polymorphic and 9 unique bands with percentage of polymorphism (100%). In the end, the primer ISSR14 primer revealed 20 bands divided into 8 polymorphic and 12 unique bands with a percentage of polymorphism (100%) in table 4 and Figure 3.

These results in table (4) were confirmed by (Rayan *et al*., 2010) which found genetic differences using doses (0, 1, 2, 4 and 8 KR) of gamma rays. (Ferreira *et al.,* 2009) found genetic differences using doses (0.0, 10, 20, 30, 40 & 50) Gy of gamma rays from *Ficus carica*. (Sukhjit Kaur 2015) show the effect of mutagens on regeneration and growth of *in vitro* grown epicotyl segments of rough lemon seedlings (*Citrus jambhiri* Lush.) The dose required to kill half of the tested population corresponded to 35Gy for gamma radiation. The number of days taken for regeneration increased with increasing dose of gamma irradiation. (Mahmoud *et al*., 2016) observed that at the molecular level, using 7 primers ISSR technology allowed measuring the genetic distance, relationship, and similarity between the selected mutants of banana lines compared with the untreated plantlets. Sixty-eight ISSR amplified bands were obtained (ranging from 237 to 1591 bp). The total number of polymorphic bands was 17 bands (25%polymorphism) while the total number of monomorphic bands was (75%monomorphism). (Ghazala *et al*., 2017) were studied of level genetic profile for investigation and exploitation of the genetic diversity of *Ficus palmata*, 25 ecotypes of *Ficus palmata* in AJK were analyzed using ISSR (Inter simple sequence repeats) markers. Phylogenetic distance estimates revealed that the ecotypes expressed common heritage for their phylogenetic relationship with a considerable genetic diversity among them as well.

Dendrogram showed that produced tissue culture (9) and mother plant (10) lines for plant (*Ficus carica subsp rupestris*) clustered together, as well as produced tissue culture (11) and mother plant (12) lines for plant (*Ficus palmata*) clustered together. There is genetic stability between mother plant and tissue culture products for plants (*Ficus palmata* & *Ficus carica subsp rupestris*) Fig. (4).

These results were confirmed by (Alizadeh &Singh 2009) they are studying genetic stability between mother plant and tissue culture products, but none of the primers showed polymorphism among micropropagated plantlets and their respective mother plants. The profiles generated based on the two marker systems were found to be highly uniform and monomorphic. Cluster analysis further confirmed the genetic stability of micropropagated plantlets. The molecular analyses precisely proved the production of genetically stable grape plantlets. (Dessoky *et al.,* 2016) studying the genetic homogeneity of the micro-propagated plants was evaluated by molecular analysis using nine randomly selected 1-year-old fig plants along with the mother plant. A total of six RAPD primers and five inter-simple sequence repeat (ISSR) primers produced a total of 109 resolvable, reproducible, and scorable bands ranging from 250 to 1550 bp in size. Among these bands, 103 bands were monomorphic (94.5%) and 6 bands were polymorphic (5.5%). (EL-shaer *et al*., 2016) found genetic differences using doses (50 and 100 Gy) of gamma rays of *Moringa oleifera*.

Effect of radiation on secondary compounds in callus of *Ficus palmata* by GC-MS Analysis.

The acetyl acetate extract of the callus leaves was treated with four doses of gamma radiation (20, 30 and 40 Gy) as compared to the control. Gamma radiation doses induced an increase in the total secondary metabolites in the callus of *Ficus palmata* by increased irradiation doses. showed twenty-four peaks from the GC-MS chromatogram in gamma radiation treatments of 20 Gy, while showed thirty-two peaks from the GC-MS chromatogram in gamma radiation treatments of 30 Gy and showed thirty-two peaks from the GC-MS chromatogram in gamma radiation treatments of 40 Gy. All three doses produced the highest as compared to the non-irradiated control (twenty-two peaks) in the table (5,6,7&8). We used 1.0 gram of callus fresh weight (g/explant) extracted with acetyl acetate. This analysis was performed to study the effects of gamma rays on secondary compounds of (*Ficus palmata*) the best doses of gamma 30 &40 Gray, as they increased the secondary compounds of the plant. These results were confirmed by (Abubakar *et al*., 2016) studying GC-MS Analysis of ethyl acetate extract of *Ficus sycomorus*. EL-shaer *et al*., 2016 study, the chemical composition was studied by means of GC-MS analysis under the effect of gamma radiation on *Moringa oleifera*.

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Table 1: initiation of callus (from leaves and stem) Medium.

Component	Concentration (g/liter)
media M.S (Murashige & Skoog)	4.4 g/l
Sucrose	30 g/l
Agar	6 g/l
Ph medium	5.7
Mio inositol	0.1 g/l
Growth	2 mg/l 2,4 – D + 0.2 Kin or
Regulators	4 mg/l 2,4-D + 0.4 Kin
Citric acid	200 mg/l
Ascorbic acid	200 mg/l
Control	0,0

Table 2: presenting the eight primers were initially used with four doses gamma ray on *Ficus palmata* & *Ficus carica subsp rupestris* to detect polymorphisms and show names and sequences of the eight primers used in this experiment.

No.	Code	Sequence
1	ISSR- 1	5'-AGAGAGAGAGAGAGAGYC-3'
2	ISSR- 3	5'-ACACACACACACACACYT-3'
3	ISSR- 5	5'-GTGTGTGTGTGTGTGTYG-3'
4	ISSR- 7	5'-CGCGATAGATAGATAGATA-3'
5	ISSR- 11	5'-CACACACACACACACARG-3'
6	ISSR- 12	5'-ATGATGATGATGATGATG-3'
7	ISSR- 13	5'-TAT ATA TAT ATA TAT AC-3'
8	ISSR- 14	5'-CTC TCT CTC TCT CTC TT-3'

Table 3: Percentage of survived explants, callus formation as revealed by culturing *Ficus palmata* leaf on three different combinations of growth regulators after five weeks

Concentration of plant growth regulators in MS medium	Formation of callus %	Survival of explant %	Days to callus initiation
Control	74	68	58
2 mg/l 2,4 – D + 0.2 Kin	91	100	31
4 mg/l 2,4-D + 0.4 Kin	77	81	49

Table 4: Polymorphism, monomorphic bands, polymorphic without unique, unique bands, polymorphic with unique bands, total number of bands and their percentage as detected by ISSR marker for *Ficus palmata* & *Ficus carica subsp rupestris.*

Primer Name	Monomorphic Bands	Polymorphic (without unique)	Unique Bands	Polymorphic (with unique)	Total number of bands	Polymorphism (%)	Mean of band frequency
ISSR1	1	16	18	34	4 35	1 97.143%	1 0.186
ISSR3	0	29	14	43	43	100.000%	0.178
ISSR5	0	14	10	24	24	100.000%	0.191
ISSR7	0	31	22	53	53	100.000%	0.160
ISSR11	0	15	9	24	24	100.000%	0.229
ISSR12	0	10	7	17	17	100.000%	0.152
ISSR13	0	15	9	24	24	100.000%	0.191
ISSR14	0	8	12	20	20	100.000%	0.196
Total	1	138	101	239	240		2,483

Percentage (%) of polymorphism = (No. of polymorphic (unique) bands ÷ Total bands) X 100.

Table 5: Phyto-components identified for callus leaves of *Ficus palmata* (control).

RT	Compound Name	Area %	Molecular Formula	Molecular Weight
20.11	1,4-benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl)-	25.79	C13H18O2	206
20.11	2h-1-benzothiopyran, 7-methoxy-2,2-dimethyl-	25.79	C12H14OS	206
20.11	3,4-dihydro-2h-1,5-(3"-t-butyl)benzodioxepine	25.79	C13H18O2	206
20.11	5-methyltricyclo[6,2,1.0(2,7)]undeca-4,9,dien-3,6,-diol	25.79	C13H18O2	206
20.11	15-methyltricyclo[6.5.2(13,14).0(7,15)]pentadeca-1,3,5,7,9,11,13-heptene	25.79	C16H14	206
23.30	Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester	16.60	C17H30O4	298
23.30	10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienyl acetate	16.60	C17H30O4	298
23.30	Methyl (3-oxo-2-pentylcyclopentyl)acetate #	16.60	C13H22O3	226
23.30	Ethanol, 2-(9-octadecenyloxy)-, (z)-	16.60	C20H40O2	312
23.45	1-(4-isopropylphenyl)-2-methylpropyl acetate	14.29	C15H22O2	234
23.45	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	14.29	C15H26O	222
23.45	5-(7a-isopropenyl-4,5-dimethyl-octahydro-inden-4-yl)-3-methyl-pent-2-enal	14.29	C20H32O	288
28.96	9-octadecenoic acid (z)-	14.81	C18H34O2	282
28.96	Dotriacontane	14.81	C32H66	450
28.96	7-Methyl-Z-tetradecen-1-ol acetate	14.81	C17H32O2	268
28.96	Isochiapin b	14.81	C19H22O6	346
28.96	Isochiapin b %2<	14.81	C19H26O6	350
30.76	Ethylene brassylate	28.51	C15H26O4	270
30.76	9-octadecenoic acid (z)-	28.51	C18H34O2	282
30.76	E-9-Tetradecenoic acid	28.51	C14H26O2	226
30.76	2,2,3,3,4,4 hexadeutero octadecanal	28.51	C18H30D6O	274
30.76	cis-13-Eicosenoic acid	28.51	C20H38O2	310

Table 6: Phyto-components identified for callus of *Ficus palmata* (20 Gy).

Rt	Compound name	Area %	Molecular formula	Molecular weight
20.03	1,4-benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl)-	5.77	C13h18o2	206
20.03	3,4-dihydro-2h-1,5-(3"-t-butyl)benzodioxepine	5.77	C13h18o2	206
20.03	15-methyltricyclo[6.5.2(13,14).0(7,15)]pentadeca-1,3,5,7,9,11,13-heptene	5.77	C16h14	206
20.03	5-methyltricyclo[6,2,1.0(2,7)]undeca-4,9,dien-3,6,-diol	5.77	C13h18o2	206
20.03	2,4-di-tert-butylphenol	5.77	C14h22o	206
37.95	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	6.00	C23h32o2	340
37.95	4h-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	6.00	C18h16o7	344
37.95	4h-1-benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	6.00	C27h30o16	610
37.95	9-octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	6.00	C28h44o4	444
44.21	Isochiapin b	24.00	C19h22o6	346
44.21	Isochiapin b %2<	24.00	C19h26o6	350
44.21	2-hydroxy-3-[(9e)-9-octadecenoyloxy]propyl (9e)-9-octadecenoate #	24.00	C39h72o5	620

Table 7: Phyto-components identified for callus leaves of *Ficus palmata* (30 Gy).

Rt	Compound name	Area %	Molecular formula	Molecular Weight
20.12	Phenol, bis(1,1-dimethylethyl)-	7.39	C14h22o	206
20.12	2,4-di-tert-butylphenol	7.39	C14h22o	206
20.12	Phenol, 2,4-bis(1,1-dimethylethyl)-	7.39	C14h22o	206
20.12	Phenol, 3,5-bis(1,1-dimethylethyl)-	7.39	C14h22o	206
29.09	Pentadecanoic acid, 14-methyl-, methyl ester	12.37	C17h34o2	270
29.09	Hexadecanoic acid, methyl ester	12.37	C17h34o2	270
32.44	9-octadecenoic acid (z)-	4.69	C18h34o2	282
32.44	Oleic acid	4.69	C18h34o2	282
32.44	Trans-13-octadecenoic acid	4.69	C18h34o2	282
32.44	9,12-octadecadienoyl chloride, (z,z)-	4.69	C18h31clo	298
38.04	4h-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	8.96	C18h16o7	344
38.04	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	8.96	C23h32o2	340
38.04	9-octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	8.96	C28h44o4	444
44.20	Isochiapin b	6.45	C19h22o6	346
44.20	Isochiapin b %2<	6.45	C19h26o6	350
44.20	Oleic acid, 3-(octadecyloxy)propyl ester	6.45	C39h76o3	592
44.24	4h-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	5.40	C18h16o7	344
44.65	2-hydroxy-3-[(9e)-9-octadecenoyloxy]propyl (9e)-9-octadecenoate #	16.77	C39h72o5	620
44.65	Dotriacontane	16.77	C32h66	450
44.86	4h-1-benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	37.97	C27h30o16	610

Table 8: Phyto-components identified for callus leaves of *Ficus palmata* (40Gy).

Rt	Compound name	Area %	Molecular formula	Molecular weight
19.95	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	2.23	C15h24o	220
19.95	Butylated hydroxytoluene	2.23	C15h24o	220
19.95	Ethanone, 1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-4h-cyclohepta[b]furan-5-yl)-	2.23	C14h20o2	220
29.09	Cyclopropane butanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	2.41	C25h42o2	374
29.09	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	2.41	C20h38o2	310
29.09	13,16-octadecadiynoic acid, methyl ester	2.41	C19h30o2	290
38.04	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	21.56	C23h32o2	340
38.04	Baimuxinal	21.56	C15h24o2	236
38.04	3-methoxymethoxy-3,7,16,20-tetramethyl-heneicosa-1,7,11,15,19-pentaene	21.56	C27h46o2	402
43.50	4h-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	6.82	C18h16o7	344
43.50	Isochiapin b	6.82	C19h22o6	346
43.50	Isochiapin b %2<	6.82	C19h26o6	350
43.50	4h-1-benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	6.82	C27h30o16	610
43.62	Ethyl iso-allocholate	3.40	C26h44o5	436
43.94	Glycidyl oleate	14.46	C21h38o3	338
43.94	Oleic acid, 3-(octadecyloxy)propyl ester	14.46	C39h76o3	592
44.17	2-hydroxy-3-[(9e)-9-octadecenoyloxy]propyl (9e)-9-octadecenoate #	23.18	C39h72o5	620

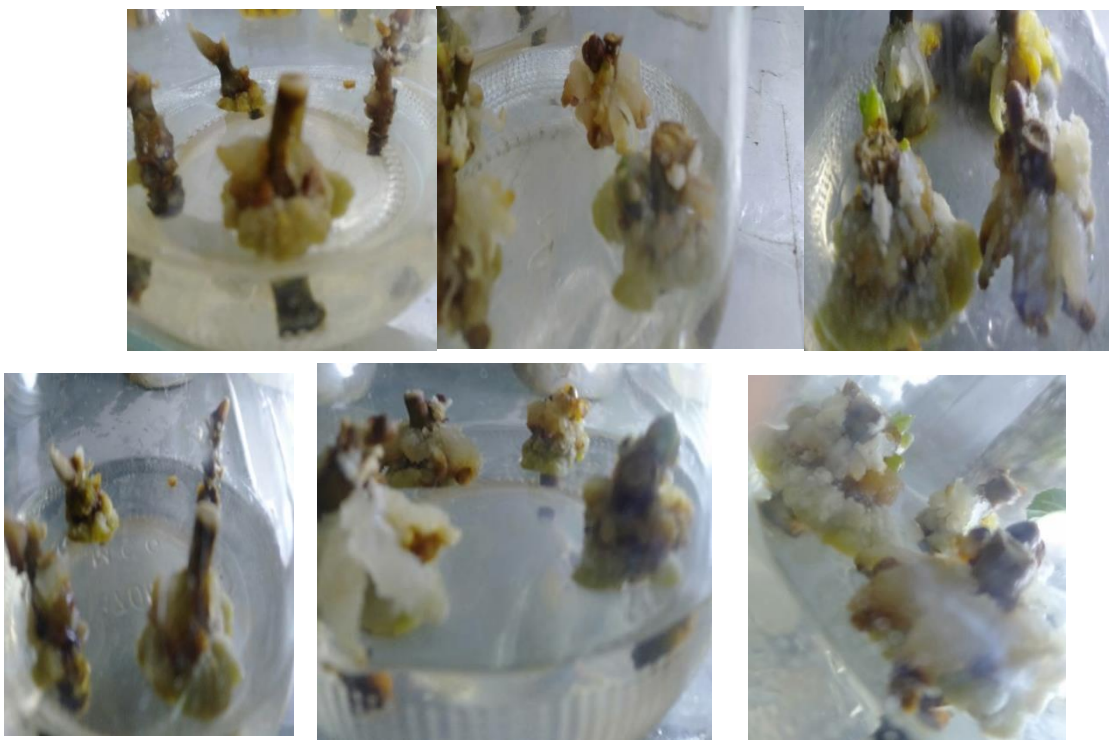

Figure 1: Effect of different combinations of Kin and 2, 4-D on callus induction from Stem of *Ficus carica supsp rupestris* & *Ficus palmata.*

Figure 2: Effect of the different doses of gamma radiation on callus size and fresh weight (g) of *Ficus carica subsp rupestris* & *Ficus palmata.*

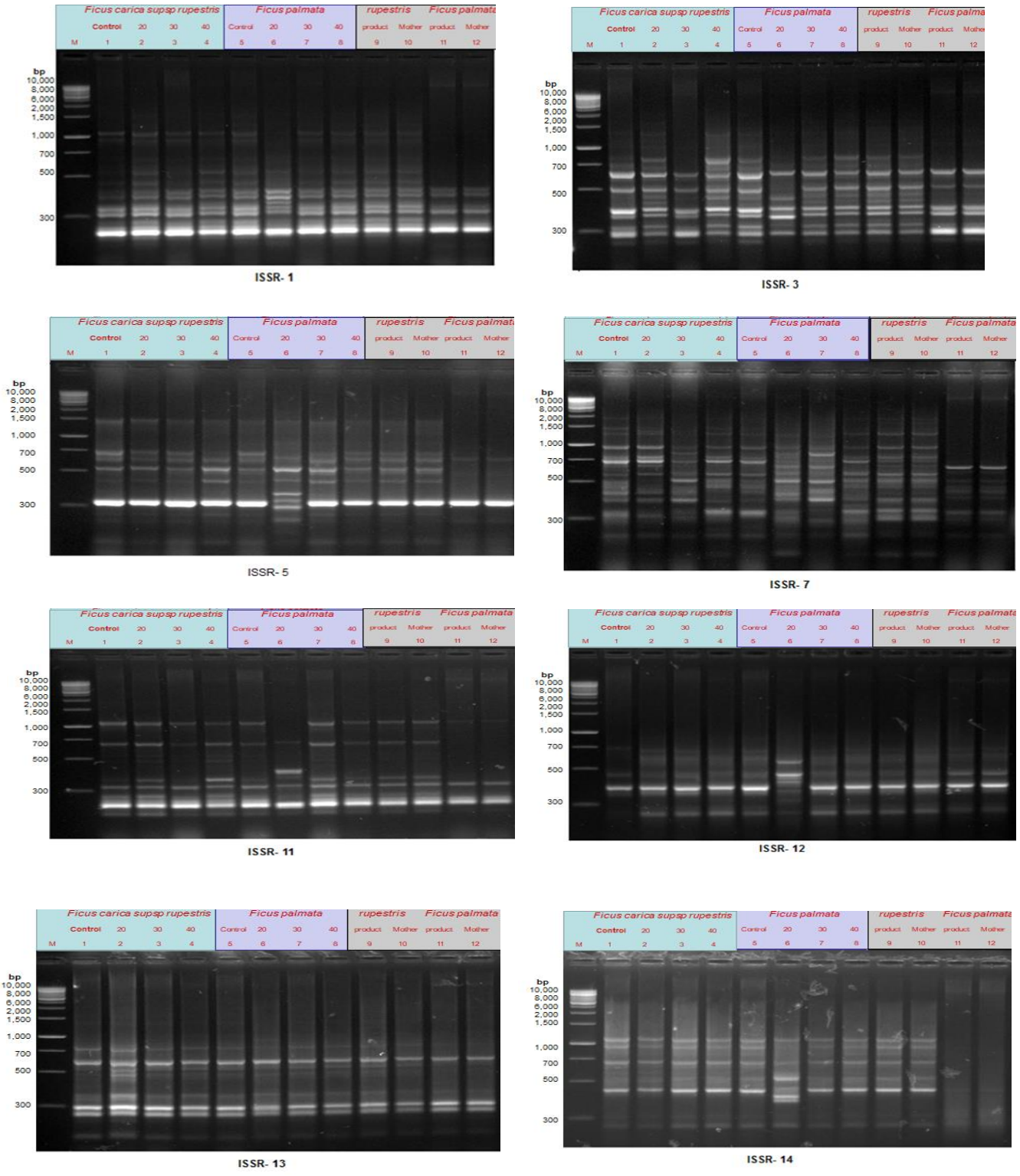

Figure 3: DNA polymorphism of the four doses of gamma radiation control, 20, 30 and 40 Gy in *Ficus carica subsp rupestris* & *Ficus palmata* and comparison with their mother plants and plants produced tissue culture amplified with primers ISSR1, ISSR3, ISSR5, ISSR7, ISSR11, ISSR12, ISSR13 and ISSR14.

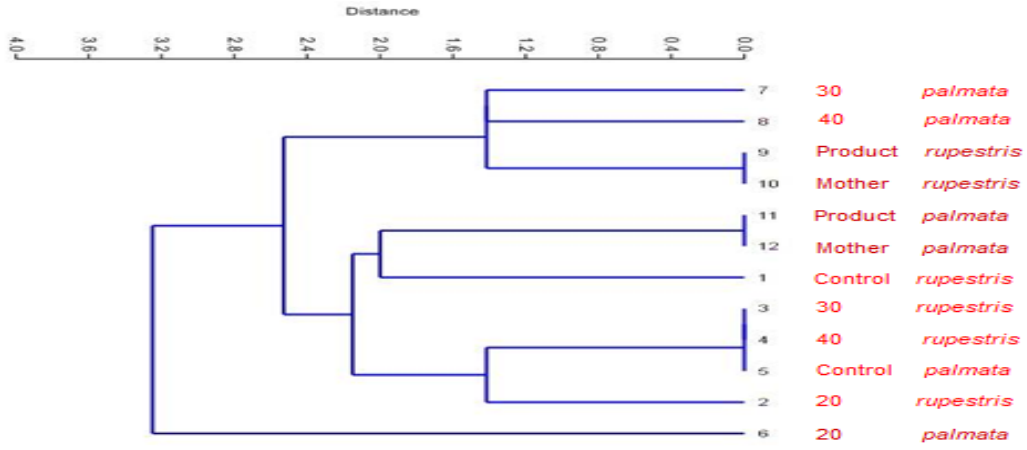

Figure 4: Dendrogram for 12 lines of *Ficus palmata* & *Ficus carica subsp rupestris* induced via using four doses of gamma radiation control, 20, 30, 40 Gy and comparison by mother plant with the plants produced tissue culture by primers ISSR1, ISSR3, ISSR5, ISSR7, ISSR11, ISSR12, ISSR13 and ISSR14.

دراسات وراثية جزيئية على نباتات التين البري (الحماط) تحت تاثير أشعه جاما باستخدام تقنيات مزارع الأنسجة

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الملخص العربى

تستخدم نباتات التين البري (الحماط) كدواء وتستخدم للحد من خطر الإصابه بالسرطان وأمراض القلب. وأظهر الفحص الكيميائى النباتى لمستخلصات نباتات التين البرى وجود القلويدات ، التانينات ، الفلافونيدات ، الترينويدات ، الجليكوسيدات. وقد أجريت هذه الدراسة لدراسة تأثير أشعه جاما بجرعات صفر، 20، 30 و40 جراى على إنتاج الكالوس والمركبات الثانوية من خلال تقنيه زراعة الأنسجة. وقد تحقق أعلى تشكل للكالوس بزراعة الأوراق والعقل الساقية لنباتات *Ficus palmata* & *Ficus carica subsp rupestris* على وسط موراشيجى & سكوج مضافا اليها 2 ملجم/لتر 2,4-D، Kin0.2. على المستوى الجزيئى تم تحديد التباينات الحادثة على مستوى الDNA للكالس المعرض للإشعاع والمقارنة مع الكنترول وأجرى هذا التحليل باستخدام تقنيه ISSR باستخدام 8 بادئات وأظهرت النتيجة أن إجمالي الحزم الناتجة (240) منها (239) حزمه متباينة بنسبة تباين (99.58%). ووجد أيضا أن هناك ثبات وراثي بين النباتات الام والنباتات الناتجة من زراعة الأنسجة بالنسبة لنباتات *Ficus carica subsp rupestris* & *Ficus palmata* . تم دراسة التركيب الكيميائي لنباتي *Ficus palmata* باستخدام تحليل الGC-MS تحت تأثير أشعه جاما وكانت أفضل الجرعات 30 و40 جراى حيث أدت الى زيادة المركبات الثانوية لنبات. Ficus palmate

الكلمات الاسترشادية: تقنية التكرارات البسيطة الترادفية الداخلية، تفاعل البوليمراز المتسلسل، فيكس بالماتا, فيكس كاريكا تحت نوع روبسترس, أشعة جاما, إنتاج الكالس.