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Research Article
Morphological and Genetic Variations of Micropropagated Paulownia Hybrid (*P. elongata* X *P. fortunei*) Using Gamma Irradiation

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Abstract

Background and Objective: Paulownia is an ornamental tree characterized by a fast-growing and short-rotation woody crop. This study aimed to detect the effect of different doses of gamma rays on morphological and DNA variations of Paulownia cultured *in vitro*. **Materials and Methods:** Micropropagated plants on MS medium supplemented with BA (0.2 mg L^{-1}), kinetin (0.1 mg L^{-1}) and IBA (0.1 mg L^{-1}) were irradiated with different 7 doses of gamma rays (0, 5, 10, 15, 20, 25 and 30 Gray). The genetic variations among the irradiated and un-irradiated Paulownia shootlets were determined using ISSR and RAPD techniques. **Results:** The ability of explants to survive was increased with 5 Gy at the highest percent (100%) for the second and third subcultures, while 10 Gy caused the highest shootlets proliferation (2.33) for the same subcultures. The 20 Gy of gamma radiation caused the highest rooting percent during the three consecutive subcultures. Photosynthetic pigment contents (Chlorophylls a, b and carotenoids) were increased at all exposure doses except at the highest dose (30 Gy). Ten ISSR primers generated in total 76 scorable fragments of which 46 (60.5%) were polymorphic. Meanwhile, a total of 75 bands were generated from five RAPD primers, 50 bands (66.6%) were polymorphic. Thus, the detected DNA polymorphism by ISSR and RAPD analysis offered a useful molecular marker for the identification of irradiated Paulownia shootlets with different doses of gamma rays. **Conclusion:** This study demonstrated that 10 Gy dose of γ-rays induced more morphological and genetic variations in Paulownia shootlets.

Key words: Paulownia, micropropagation, gamma radiation, ISSR, RAPD

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Paulowniaceae family includes a genus of Paulownia which comprises many species with similar properties. Most species of Paulownia are among the fastest-growing trees in the world as the harvesting begins within 8-10 years. In recent years, the concern of industrial uses of this genus is increasing due to its economic feasibilities as a raw material for the wood industry and bioenergy source[1]. Paulownia produces a soft, lightweight wood with great machining and finishing characteristics[2], which widely used for manufacturing furniture, doors, windows and musical instruments as well as paper pulp[3-5]. Some species of Paulownia have also ornamental uses[6,7] and afforestation, where it grows well in a wide range of soil types and tolerate poor soil[8]. Due to these characteristics, among the most important forestry crops in the world are Paulownia species.

Applications of radiation are often used for developing plant varieties that possess agricultural and economic importance with high potential production. Gamma radiation and its applications in agriculture science are extremely important physical mutagens for mutation breeding to improve plant traits and develop genetic variabilities, such as resistance to cold, saltiness and diseases[9].

In vitro cultures mutations using gamma rays is the most commonly mutagenic agent, that has been reported to affect differently the morphology, biochemistry, anatomy and physiology of plants depends on the level of irradiation[10]. These effects involved changes in metabolism and plant cellular structure, such as a change in photosynthetic pigments and several physiological metabolites[11,12]. In this respect, gamma rays have a higher degree of accuracy, adequate reproducibility and deep penetrating vigour into the biological matter which can cause a higher number of variations in physiochemical composition[13]. The biological effect of gamma rays based on molecules or atoms interaction within the cell, particularly water, to produce free radicals[14]. Therefore, variations induction should be confirmed by mutant screening and characterization[15].

DNA-based genetic markers assays appear to provide the means to give beneficial information on DNA polymorphism, diversity, genetic stability and the identification and screening of mutant plants, such as *Sophora davidii*[16]. Therefore, molecular markers allow a direct comparison of the effects of genotypes at the DNA level. Random amplified polymorphic DNA (RAPD) as well as Inter simple sequence repeat (ISSR) profiles are dominant markers, which widely applied in fingerprinting, also the characterization of markers related to beneficial traits[17,18], that rapidly being used by the research community in various fields of plant improvement[14]. The RAPD analysis hence can be used for the detection of DNA alterations after the influence of mutagenic agents[19], DNA polymorphism detected by ISSR and RAPD analysis offered a useful molecular marker for the identification of mutants in gamma radiation treated *Helichrysum bracteatum* L. plant[20]. The experimental work was carried out to detect the effect of different doses of gamma rays on morphological characteristics of Paulownia cultured *in vitro* also, DNA variation among the irradiated and un-irradiated plants using ISSR and RAPD techniques.

MATERIALS AND METHODS

Study area: The present study was conducted at Tissue Culture Technique Laboratory, Department of Ornamental Plants and Woody Trees, National Research Center (NRC), Egypt, during years 2019 and 2020 to investigate the response of some morphological and genetic variations of micropropagated Paulownia hybrid (*P. elongata*× *P. fortunei*) shootlets that irradiated with different doses of gamma rays.

Plant material and surface sterilization: Nodal explants were collected from P. hybrid (*P. elongata*× *P. fortunei*) tree maintained at the Faculty of Agriculture, Ain Shams University, Shubra Al Khaymah, Egypt, a source of *in vitro* established plantlet. The nodal segments (15-20 cm length) were prepared by washing under tap water with a few drops of liquid soap for 1 h and then rinsed three times in sterile demineralized water. Sterilization surface of explants was performed under aseptic conditions in a laminar airflow hood. Initially, for 30 sec in 70% (v/v) ethanol solution (C_2H_5OH) followed by 15% (v/v) commercial sodium hypochlorite solution (NaOCl 5.25%) and one drop of tween 20 ($C_{58}H_{114}O_{26}$) with shaking for 20 min then rinsed three times with autoclaved distilled water. After that, the explants surface immersed in mercuric chloride (Hg_2Cl) 0.2% for 7 min finally, the nodal segments were rinsed three times with autoclaved distilled water.

Culture medium and incubation condition: Murashige and Skoog[21] Basal Medium (MS) at full strengths supplemented with sucrose at 2.5% (w/v) and growth regulators: 6-furfuryl amino Purine at 0.2 mg L^{-1}, kinetin at 0.1 mg L^{-1} and indole-3-butyric at 0.1 mg L^{-1} was solidified with agar at 0.7% (w/v) and adjusted to 5.7±0.2 pH which was autoclaved at 121°C for 15 min and 1.2 kg cm^{-2}. All cultures of the experiment were incubated under photoperiod 16 hrs of fluorescent light with 30 µmol m^2 sec^{-1}.

Table 1: List of primers and DNA fingerprint profile of gamma-irradiated Paulownia hybrid (*P. elongata*× *P. fortunei*)

Marker	Primer	Sequence (5'-3')	GC (%)	Size range (bp.)	TAB	NMB	NPB	PPB
ISSR	UBC-807	AGA GAG AGA GAG AGA GT	47	250-900	7	4	3	43
	UBC-808	AGAGAGAGAGAGAGAGC	52	200-600	6	4	2	33
	UBC-811	GAGAGAGAGAGA GAGAC	52	230-1200	10	3	7	70
	UBC-812	GAGAGAGAGAGAGAGAA	64	350-800	9	4	5	55.5
	UBC-817	GTG TGT GTG TGT GC	57	400-1500	7	5	2	28.5
	UBC-825	CACACACACACACACAA	47	400-1200	7	2	5	71.4
	UBC-864	ACA CAC ACA CAC ACA CT	47	300-800	8	1	7	87.5
	HB09	ATG ATG ATG ATG ATG ATG	33	280-1250	7	2	5	71.4
	HB-13	GTG GTG GTG GC	72	250-800	8	2	6	75
	HB-15	GAG GAG GAGGC	72	150-1000	7	3	4	57
	Total	-	-	-	76	30	46	-
	Mean	-	-	-	7.6	3	4.6	59.23
RAPD	OPB-15	GGAGGGTGTT	60	100-2500	14	1	13	93
	OPB-18	CCACAGCAGT	60	150-2000	14	4	10	71.4
	OPC-19	GTTGCCAGCC	70	220-1400	18	8	10	55.5
	OPD-16	AGGGCGTAAG	60	130-2500	13	4	9	69
	OPF-01	ACGGATCCTG	60	180-2500	16	8	8	50
	Total	-	-	-	75	25	50	-
	Mean	-	-	-	15	5	10	67.78

TAB: Total amplified bands, NMB: Number of monomorphic bands, NPB: Number of polymorphic bands, PPB: Percentage of polymorphic bands

Procedure layout: The shootlets that were obtained after 2 months from cultured explants on the prepared medium were exposed to different seven doses of gamma rays (0, 5, 10, 15, 20, 25 and 30 Gray). The irradiated shootlets were sub-cultured three repeated times on the same established medium. *In vitro* proliferated shootlet parameters were represented in the survived explants (%), shootlets number/explant, shootlets length (mm), leaves number/shootlets as well as the *in vitro* root growth parameters (rooting percentage %, roots number/shootlets and root length mm) for each sub-culture were recorded after two months.

Gamma irradiation: The irradiation facility was carried out at Atomic Energy Commission-united irradiation-Gamma, Nasr city, Egypt, using Cesium 137 as a gamma rays source at a rate of 0.658 rad sec^{-1}.

Determination of photosynthetic pigments content: Chlorophyll a, b and carotenoids pigments contents (mg 100 g^{-1} F.W.) were determined according to the method described by Smith and Benitez[22].

DNA extraction: Total genomic DNA has extracted from fresh leaves tissues of gamma treated and untreated Paulownia shootlets using the Cetyltrimethyl Ammonium Bromide (CTAB) procedure as described by Doyle and Doyle[23].

PCR amplification conditions: Two DNA marker techniques (RAPD and ISSR) were used to detect changes in the genomic DNA of exposed Paulownia hybrid (*P. elongata*× *P. fortunei*) shootlets to different seven doses of gamma radiation given in Table 1. The PCR amplification reactions were performed within 25 µL total volume containing 12.5 ng templates DNA, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 µM primers (Operon Technologies Inc., CA and the USA), 200 µM dNTP mix and 1.0 U of *Taq* DNA polymerase (Promega Corporation). However, PCR amplifications were performed using (Techni TC-512) PCR thermo-cycle with initial denaturation at 94°C for 4 min, followed by 40 cycles of amplification with denaturation at 94°C for 1 min, annealing at 38-45°C for 1 min, extension at 72°C for 2 min and a final extension at 72°C for 10 min. The amplified products were electrophoresed on 1.5% agarose gel containing ethidium bromide (0.5 µg mL^{-1}) using 1X TBE buffer at 100 V for 1 h. The DNA fragments were visualized under UV light and photographed using the gel documentation system to detect DNA polymorphism between the irradiated and un-irradiated Paulownia plants.

Data of PCR analysis: The data were recorded for presence (1) or absence (0) of bands for each of the primer pairs, which set in a binary matrix. According to Dice[24] coefficient of similarity was measured and a dendrogram based on similarity coefficients were generated by using unweighted pair group method with arithmetic mean (UPGMA), using "IBM SPSS Statistics" software for Windows (version 25).

Statistical analysis: A randomized complete block design was used with three replicates. Analysis of variance of the obtained data from each attribute was computed using the MSTAT Computer Program[25]. Means were compared using the Least Significant Differences test (LSD) at a 5% level of probability[26].

RESULTS AND DISCUSSION

Morphological characterization

***In vitro* shooting ability:** The tabulated data of Table 2 and 3 indicated the effect of various gamma radiation doses (0, 5, 10, 15, 20, 25 and 30 Gy) on *in vitro* shooting ability of Paulownia hybrid (*P. elongata*× *P. fortunei*) during three consecutive subcultures. Concerning the explant survival ability, data in Table 2 showed that the explants could survive to 94-100% when the shootlets were exposed to high doses (20, 25 and 30 Gy) of gamma radiation during the first and second subcultures then depressed until 66.67-89.67% in the third subculture. However, shootlets exposure to the low dose of gamma radiation (5 Gy) gradually increased the ability of explants to attain the highest percent (100%) for the second and third subcultures. Regarding shootlet proliferation, the highest value (3.00) during the first subculture was obtained with gamma exposure of 20 Gy. This value was decreased to be 1.43 and 1.50 with no significant differences between the above-mentioned values and those of control (1.20 and 1.33) as shown in Fig. 1a during the second and third subcultures as compared to that was obtained with 10 Gy Fig. 1b which caused the highest shoot proliferation/explant (2.33) during the same subcultures.

As shown in Table 3 and Fig. 1a and b, exposing the shootlets to 10 Gy led to the longest shootlets (75.00 and 60.33 mm) during the first and second subcultures, also the highest number of leaves/ shootlets (17.00 and 14.67) during the second and third subcultures as compared with control. Increasing the exposure dose of gamma radiation to 30 Gy increased the values of shootlet elongation during the third subculture and the number of leaves/ shootlet during the first subculture to the highest values (78.33 mm and 18.67, respectively) comparing to control and other treatments.

From the previously mentioned results, it can be noticed that low doses of gamma radiation (5 and 10 Gy) had a stimulation effect on *in vitro* shooting ability during the three examined subcultures while high doses had an inhibition effect except for shootlet elongation which recorded the highest value by exposed the shootlets to the highest dose of gamma radiation (30 Gy) during the third subculture. This finding was agreed with that reported by Muthusamy and Jayabalan[27], who pointed out that exposing the explants to lower gamma radiation shortened the days to shoot and root initiation. In the same trend, Hashish *et al.*[28] on *Hibiscus rosa-sinensis* and Abou Dahab *et al.*[29] on *Eustoma grandiflorum*. The stimulation effect of low radiation doses might be attributed to increasing some enzymes activity[30]. The

Table 2: Effect of gamma radiation on explant survival and shootlets proliferation of Paulownia hybrid (*P. elongata*× *P. fortunei*)

	Treatments					
	Survival (%)			Number of shootlets/explant		
γ-ray (Gy)	Sub.1	Sub.2	Sub.3	Sub. 1	Sub.2	Sub.3
0	61.33	100.00	86.67	1.17	1.20	1.33
5	82.00	100.00	100.00	1.33	1.80	1.17
10	86.67	100.00	88.67	1.10	2.33	2.33
15	80.00	89.00	76.33	1.00	1.67	1.00
20	100.00	100.00	89.00	3.00	1.43	1.50
25	100.00	100.00	66.67	1.37	1.23	1.20
30	94.33	88.33	66.67	1.57	1.27	1.43
$LSD_{0.05}$	2.67	1.82	3.96	1.02	0.86	0.74

γ-ray (Gy): Gamma radiation doses, $LSD_{0.05}$: List significant differences of means at 0.05

Table 3: Effect of gamma radiation on shootlet elongation and number of leaves/shootlet of Paulownia hybrid (*P. elongata*× *P. fortunei*)

	Treatments					
	Length of shootlets (mm)			Number of leaves/shootlet		
γ-ray (Gy)	Sub. 1	Sub. 2	Sub. 3	Sub. 1	Sub. 2	Sub. 3
0	56.00	35.33	30.00	12.67	10.33	12.00
5	62.67	38.00	31.00	13.00	14.67	12.00
10	75.00	60.33	62.33	13.33	17.00	14.67
15	44.00	37.33	69.00	11.33	13.67	11.67
20	46.00	50.67	72.67	11.33	11.67	12.33
25	34.67	47.67	60.00	11.33	12.33	11.67
30	48.67	55.67	78.33	18.67	12.00	8.33
$LSD_{0.05}$	4.00	3.28	5.35	2.93	2.75	2.48

γ-ray (Gy): Gamma radiation doses, $LSD_{0.05}$: List significant differences of means at 0.05

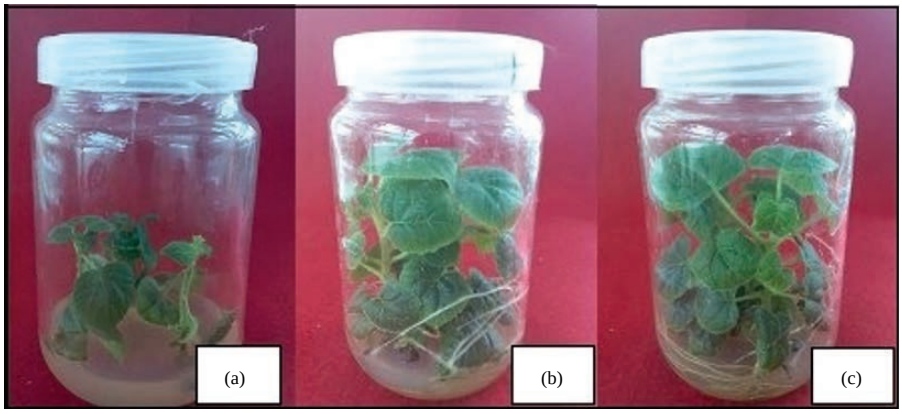

Fig. 1(a-c): Effect of gamma radiation on shootlets proliferation and rooting ability of Paulownia hybrid (*P. elongata*× *P. fortunei*) during the third subculture
(a) Control (0 Gy), (b) Shootlets exposed to 10 Gy and (c) Shootlets exposed to 20 Gy

Table 4: Effect of gamma radiation on *in vitro* rooting ability of Paulownia hybrid (*P. elongata*× *P. fortunei*)

	Treatments								
	Rooting (%)			Number of roots/shootlet			Length of roots (mm)		
γ-ray (Gy)	Sub. 1	Sub. 2	Sub. 3	Sub. 1	Sub. 2	Sub. 3	Sub. 1	Sub. 2	Sub. 3
0	75.33	67.00	16.67	1.67	2.67	1.00	93.33	69.33	26.00
5	75.00	59.33	50.00	2.00	3.33	2.67	50.33	92.33	71.33
10	66.67	75.00	77.00	3.33	4.00	1.00	46.67	85.00	82.67
15	33.33	44.33	66.67	2.67	2.67	3.67	85.00	85.67	98.67
20	91.67	82.00	89.00	4.00	3.00	2.00	65.00	100.00	117.70
25	32.00	45.00	44.33	1.67	2.67	1.67	53.33	82.67	73.33
30	66.67	56.00	27.33	1.33	1.67	1.33	80.00	75.00	43.67
$LSD_{0.05}$	4.19	2.99	3.39	0.84	1.09	0.61	5.67	3.48	3.74

γ-ray (Gy): Gamma radiation doses, $LSD_{0.05}$: List significant differences of means at 0.05

inhibition effect of high gamma radiation might cause an interruption of auxin synthesis as the earlier finding that was reported by Gordon[31].

***In vitro* rooting ability:** The recorded results in Table 4 and Fig. 1c revealed that exposure of shootlets to 20 Gy of gamma radiation resulted in the highest rooting percent (ranged from 82-91.67%) during the three consecutive subcultures. Moreover, the same dose (20 Gy) led to the highest numbers of roots (4.00) during the first subculture and the same value (4.00) was also obtained with 10 Gy in the second subculture. Using 15 Gy of gamma radiation produced the highest root number (3.67) during the third subculture. While, the longest roots (93.33, 100 and 117.70 mm, respectively) were observed with control (un-irradiated shootlets) during the first subculture and 20 Gy treatments during the second and third subcultures, consecutively, compared with other treatments. Generally, increasing gamma radiation doses had an inhibition effect on rooting ability as mentioned by several researchers[32,12,33], which attributed to the sensitivity of gamma radiation that affects the amount of endogenous auxin and cytokinin and causes disruption of hormonal balance.

Photosynthetic pigment: Concerning the effect of different gamma radiation doses, leaves photosynthetic pigment contents (Chl. a, b and carotenoids) were increased at all exposure doses except for the highest dose (30 Gy) which reduced these values. The maximum values (854.2, 274.7 and 1047.5 mg 100 g^{-1} F.W., respectively) were recorded for low gamma radiation dose (5 Gy) as compared to control and other doses in Fig. 2.

It could also be noticed that these values of pigments content were in an opposite relation with increasing the dose of gamma radiation. This degradation may be due to the extreme sensitivity of chloroplasts to gamma radiation compared with other cell organelles[34], similar results were found on *Paulownia tomentosa* pigment contents which

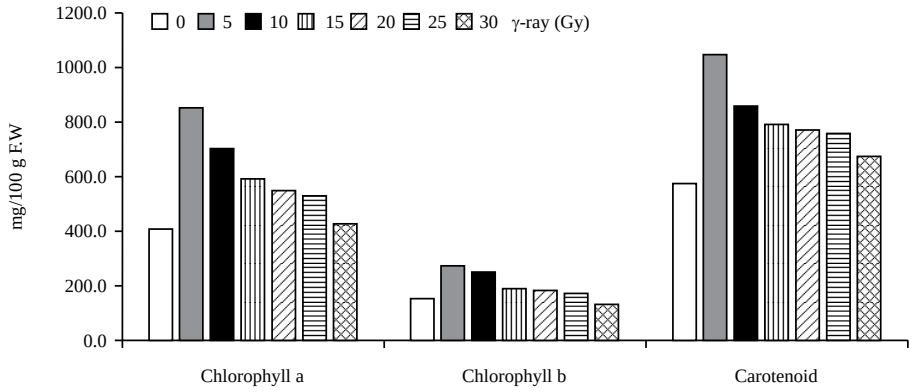

Fig. 2: Effect of gamma radiation on Paulownia shootlets pigments contents (Chlorophyll a, b and carotenoids)

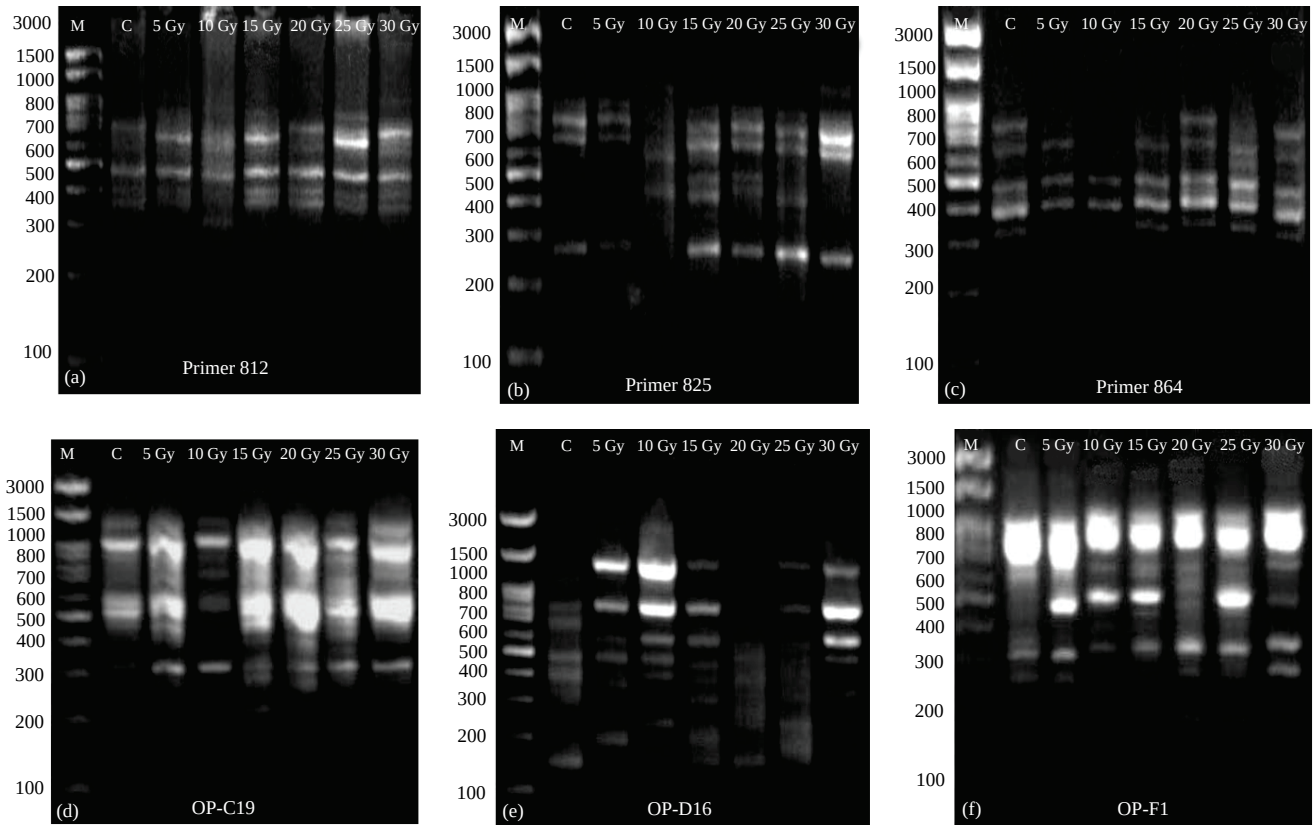

Fig. 3(a-f): ISSR- RAPD profiles of Paulownia hybrid irradiated with different six doses of gamma rays

(a) Agarose electrophoresis of ISSR -PCR assay of UBC-812 Primer, (b) ISSR profile of primer UBC-825, (c) ISSR profile of UBC-864 Primer, (d) Agarose electrophoresis of OP-C19 Primer, (e) RAPD profiles of OP-D16 Primer and (f) The amplified fragment of OP-F1 Primer, Lane M: DNA ladder 100 bp., lane C: Control, lanes 5 -30 Gy: PCR products of irradiated shootlets with six doses

were increased in plants irradiated with gamma rays[35]. The pigment level increases with low radiation levels exposure were obtained as a result of enzyme system activation[36].

PCR amplification for ISSR and RAPD analysis: Twenty ISSR and fifteen RAPD primers were tested out of which ten ISSR and five RAPD primers, respectively were selected finally depend on their ability to detect DNA polymorphism among the irradiated and control Paulownia shootlets.

ISSR analysis: Changes in the genomic DNA of treated Paulownia shootlets with gamma radiation were detected by

ISSR profiles using ten primers comparing with the control in Fig. 3a-c and Table 1. A total of 76 bands were scored, ranged from 150-1500 bp of which 46 were polymorphic (60.5%) and 30 bands were monomorphic (39.4%). The number of bands generated per primer varied from 6-10 which a minimum of 6 bands was generated by the primer UBC-808, while the maximum of 10 bands was recorded with UBC-811 followed by UBC-864 which produced 8 bands (Table 1). The percentage of polymorphism was ranged from 28.5-87.5%, with an average polymorphism percentage of 59.23%. The ISSR locus results appeared that, exposing Paulownia shootlets to gamma radiation-induced new bands or the absence of amplified products compared with unirradiated shootlets. A total number of 19 unique markers were identified by using ISSR primers as shown in Table 5, which ranged in size from 200-1250 bp., twelve of them were detected with the dose 10 Gy of gamma irradiation with primers [UBC-811(+500, +600 bp.), UBC-812 (+300, +510 bp.), UBC-817(+1000, +1200 bp.), UBC-825(-700 bp.), HB-15 (-300 bp.) and UBC-864 (-280, +600, -800, -900 bp.), while the remained 7 markers were detected with the dose 5 Gy (UBC-811(+500, +600 bp.)] and UBC-864 (-1250 bp.), 20 Gy [HB-13 (+200, -800 bp.)], 25Gy [UBC-825(+600 bp.)] and 30Gy [UBC-864 (-400 bp.)]. The results indicated that the 10 Gy dose of γ-rays induced more genetic variation in Paulownia hybrid (*P. elongata*× *P. fortunei*) shootlets (Fig. 3 and Table 5).

RAPD analysis: The PCR amplification using five RAPD primers gave rise to reproducible amplification products given in Table 1 and Fig. 3d-f, the results were analyzed for identifying genetic diversity in gamma rays treated plants. A total of 75 bands were scored, ranged from 100-2500 bp., of which 50 were polymorphic (66.6%) and 25 bands were monomorphic (33.3%) (Table 1). The number of bands generated per primer varied from 13 (OP-D16) to 18 (OP-C19). The percentage of polymorphism was ranged from 50% (OP-F01) to 93% (OP-B15) with an average polymorphism percentage of 67.78%. (Table 1). The results RAPD revealed that, exposing Paulownia shootlets to gamma radiation-induced new loci or the absence of bands compared with the control individuals. A total number of 24 unique markers were identified by using RAPD primers as showed in Table 5. These markers ranged in size from 100 to 2225 bp., nine of them were detected with the dose 30 Gy of gamma irradiation, eight of them with primer (OP-B15) with molecular size (+100, +200, -360, +500, -600, +700, +1300 and +2225 bp.) as well as one marker with primer (OP-F01) at +2000 bp., followed by the dose 10 Gy of gamma irradiation, seven markers were detected with primers (OP-B15) at +800 bp., (OP-B18) bp at +1100 bp., (OP-C19) at -290, -400 and -1500 bp. and (OP-D16) at +130 and -300 bp., while the remained eight markers were detected with control at Mwt. (-600 and +700 bp.), the dose 15 Gy (OP-B18 at -350 and -700 bp), 20 Gy (OP-B15 at +200 bp, OP-F01 at +490, +690 bp) and 25 Gy (OP-B18) at -1250 bp. The results indicated that two of the 30 Gy and 10 Gy doses of γ-rays induced more genetic variation in Paulownia shootlets (Fig. 3 and Table 5).

Table 5: Dose treatment characterized by positive (+) and negative (-) specific markers with their molecular sizes (bp.) and the total number of markers for each dose using ISSR and RAPD analysis

Dose treatments	Marker type	Primer	Mol. Size (bp).	No.	Marker type	Primer	Mol. Size (bp)	No.	Total number of markers
0 Gy	ISSR	-	-	-	RAPD	OPD-16	600,700	-1,+1	2
5 Gy		UBC-811	500,600	+2		-	-	-	3
		UBC-864	1250	-1		-	-	-	
10 Gy		UBC-811	500,600	+2		OP-B15	800	+1	19
						OP-B18	1100	+1	
		UBC-812	300,510	+2		OP-C19	1500,290 400	-3	
		UBC-817	1000,1200	+2		OPD-16	130,300	+1,-1	
		UBC-825	700	-1					
		UBC-864	600,280, 800,900	+1,-3					
		HB-15	300	-1					
15 Gy		-	-	-		OPB-18	350,700	-2	2
20 Gy		HB-13	200,800	+1,-1		OPB-15	200	+1	5
						OPF-01	490,690	+2	
25 Gy		UBC-825	600	+1		OPB-18	1250	-1	2
30 Gy		UBC-864	400	-1		OPB-15	100,200, 360,500 600,700,1300,2225	+2, -1,+1,-1,+1	10
						OPF-01	2000	+1	
Total		-	-	19		-	-	24	43

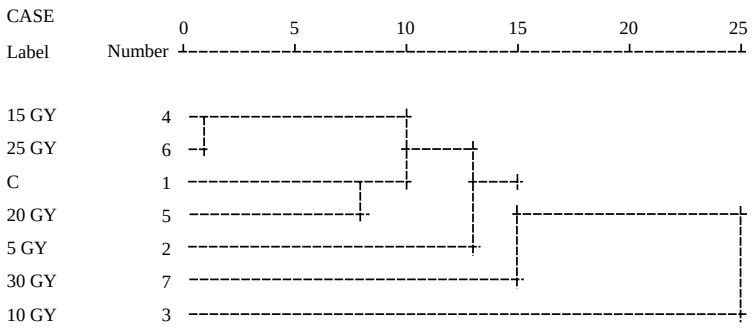

Fig. 4: Dendrogram for irradiated Paulownia hybrid and control constructed from ISSR and RAPD combined data using UPGMA and similarity matrices computed according to Dice coefficient

Table 6: Similarity index based on ISSR and RAPD combined data among gamma-irradiated Paulownia hybrid and control

Gamma dose	Control	5 Gy	10 Gy	15 Gy	20 Gy	25 Gy	30 Gy
Control	0.000						
5 Gy	0.333	0.000					
10 Gy	0.476	0.381	0.000				
15 Gy	0.286	0.286	0.619	0.000			
20 Gy	0.429	0.429	0.762	0.190	0.000		
25 Gy	0.333	0.333	0.667	0.000	0.238	0.000	
30 Gy	0.667	0.476	1.000	0.619	0.667	0.667	0.000

The banding profile of irradiated shootlets and control with different ISSR and RAPD primers is depicted in Fig. 3. In these studies, the genetic variation rate of the irradiated plant was markedly increased as evaluated by ISSR and RAPD markers, these results revealed that ISSR and RAPD markers are sensitive to the detection of genetic diversity at the DNA level. These results are in agreement with Wendt *et al.*[18], who applied the ISSR analysis to study the effect of γ-rays on the *Solanum tuberosum*. Furthermore, Mudibu *et al.*[37] detected changes in the DNA bands in γ-rays irradiated soybean plants using ISSR assay by appearance or disappearance of some loci. Further, the molecular characterization of mutants might be helpful for the set-up of an efficient mutation induction protocol[38]. The main changes observed in profiles were the appearance or disappearance of loci under γ-rays which might be considered molecular markers for radiation actions[39]. These effects of γ-rays may be correlated with structural re-arrangements in the genomic DNA caused by various kinds of DNA damages[19]. It is concluded that DNA polymorphism detected by ISSR and RAPD analysis offered a useful molecular marker for the identification of mutants in gamma radiation treated plantlets.

Genetic relationship as detected by combined data of ISSR and RAPD analysis: The genetic variations among the irradiated and control (un-irradiated) Paulownia shootlets were determined by Dice similarity coefficient to depend on ISSR and RAPD combined data given in Table 6. The genetic similarity ranged from a maximum of 1.00 (between 10 Gy and 30 Gy) to a minimum of 0.00 (between 15 Gy and 25 Gy). The UPGMA cluster analysis of the Dice similarity coefficient produced a dendrogram in Fig. 4 which showed the genetic relationship between the irradiated and control Paulownia shootlets. The analysis illustrated that γ-irradiated Paulownia shootlets and the control fell into two main clusters. The first main cluster was composed of the 10 Gy γ-Radiations, while the second cluster II involved three groups: the first group comprised of the 30GY γ-Radiation. The second group consisted of 5 Gy γ-Radiations. The third group involved 15 Gy, 25 Gy, Control and 20 Gy irradiated shootlets. According to the obtained dendrogram, 10 Gy and 30 Gy were more distant to control than to other treatments. These results agreed with those obtained by Dhakshanamoorthy *et al.*[40], who found that gamma rays induced mutants in *Jatropha curcas* using 23 RAPD primers. The proximity matrix based on RAPD analysis of EMS-induced mutants showed that three mutants were more distinct from the control.

CONCLUSION

In the present study, exposure Paulownia shootlets to 10 Gy of gamma rays induced most changes in the patterns of DNA bands using ISSR and RAPD assays in comparison with unirradiated ones. This finding was confirmed in morphological traits that represented shootlets proliferation/explant which might be due to mutation induction.

SIGNIFICANCE STATEMENT

This study discovered the potential of gamma rays that can be beneficial to enhance the proliferation rate and induce mutation of Paulownia. This study will help the researchers to uncover the critical areas of Paulownia production that can help to reduce the micro propagated pants losses that many researchers were not able to explore. Thus, a new theory on using gamma radiation may arrive at mutation that produces a huge number of the tree.

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