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THE USE OF SRAP MARKER TO DETECT THE GENETIC STABILITY OF MICROPROPAGATED *Magnolia grandiflora* L.

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ABSTRACT

This study was done in Molecular Biology and Plant Tissue Culture labs at the research center, College of Science, Duhok University, Kurdistan Region, Iraq, from August 2021 to September 2023. Shoot tips were used as an explanation in this study. Optimal sterilization was achieved when 70% (v/v) ethanol was used for 2 minutes, then explants were soaked in 2.5% (v/v) of NaOCl for 20 minutes. The better medium for initiation was MS supplied with 1.0 mgL^{-1} BA + 0.05 mgL^{-1} of NAA, which increased shoot number to (2.60), BA at 2.0 mgL^{-1} + NAA at 0.5 mgL^{-1} gave (2.60) number of nodes. At the multiplication stage, a combination of BA at 6.0 mgL^{-1} + NAA at 1.0 mgL^{-1} produced the best numbers (2.50) shoots/ explant, the highest number (2.06) of nodes produced on (MS) medium contained both BA at 2.0 mgL^{-1} + NAA at 2.0 mgL^{-1}. However, there was no significant difference between MS and WPM media regarding multiplication rate. In vitro, roots were developed when WPM contained 0.5 mgL^{-1} IBA. An autoclaved mixture of peat moss and loam at a ratio (1:0.5) (v: v) was used for plant acclimatization with an 80- 85 % survival rate. Twenty-four SRAP primers were used to test the uniformity and stability of micro-propagated plants. No polymorphism was found, indicating the genetic stability of micro-propagated plants.

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INTRODUCTION

Magnolia (Magnolia grandiflora. L), also called evergreen magnolia, bull boy, big flower, and evergreen trees. Magnolia was popularized quickly for its glossy, evergreen foliage and elegant form, and it was planted widely as an ornamental plant (ElGdwey *et al.*, 2015). The importance of magnolia is a graceful form and evergreen species with an abundant blossom, usually planted in gardens. (Cui *et al.*,2019). Decorative leaves and beautiful flowers distinguish magnolias; the colors of magnolias are varied from white to violet. Magnolias are considered valuable plants because of their foliage and showy flowers. Also, they are grown for their woody and possible pharmaceutical applications (Radomir 2012, Dimitrova *et al.*, 2021). It is known that propagation of Magnolia grandiflora L. is done by seeds, but they have a lower germinability rate (35%). Also, progeny produced by seeds has a variation, and sound characteristics of doner plants cannot be maintained well in them (Kang *et*

al.,2020). or by cutting is also tricky because of its poor ability to root, or by layering, but it is wasting time- method and very expensive.

The requirements of Magnolia for rooting facility are expensive and may not be available to shoot rooting (Sokolov *et al*., 2014; ElGwedy *et al*.,2015; Huai *et al*.,2010). Therefore, using plant tissue culture techniques to propagate Magnolia may have many advantages over traditional methods (Al-Drisi *et al*., 2022). It is considered one of the advanced applications for plant cells and tissue culture techniques to increase this species in vitro (Safana *et al*., 2022). The propagation of magnolia using tissue culture is an opportunity to improve the multiplication efficiency of these magical plants. This method should be helpful in quickly supplying a vast number of these species (Shahzad *et al*., 2017). The tissue culture technique greatly assists in addressing related challenges in Magnoliaceae (Huai *et al*.,2010). Under long conditions of in vitro, many factors such as composition of media, PGRs, and conditions of growth can induce variations in regenerated plants (Bairu *et al*.,2007 and Hussain *et al*., 2018), so the estimation of genetic stability for micro propagated plants is fundamental using different DNA markers. Sequence-related amplified polymorphism (SRAP) marker is extensively used to confirm the genetic fidelity of many plants. The study's objectives were to determine a method to reproduce the Magnolia genotype by tissue culture and analyze the genetic stability of micro-propagated plants. The study also studied the effect of different PGRs and their concentration on Magnolia micropropagation using shoot tips.

MATERIALS AND METHODS

Explants And Sterilization

The shoot tip explants used in this study were collected from (*Magnolia grandiflora* L.) plants grown in Avro City Complex, Duhok City, Kurdistan Region of Iraq, during the period from August 2021 to September 2023. Shoot tips were selected as explant sources, the explants were washed under the tap water for one hour with the addition of liquid soap every 10 minutes, and after that, rinse for 5 minutes with sterile distilled water. Under a laminar cabinet, the explants were soaked with 70% (v/v) ethanol for 2 minutes and then put in 2.5% (v/v) of NaOCl for 20 min. and they are washed with sterile distilled water 3 times and become ready for culture.

Initiation Stage

MS medium consist of several concentrations of BA (0.0, 0.5, 1.0 and 2.0 mgL^{-1}), NAA at (0.0, 0.05, 0.1 and 0.5 mgL^{-1}) and GA_3 at 2.0 mgL^{-1} was used for explant culture. 3 explants in each jar with 10 replicates were cultured and put in the growth room for 4 weeks and observations of shoots and node numbers were recorded. Browning of explants due to oxidation of phenol compounds was the major problem during the initiation stage associated with the explants and can be control by some extent, by antioxidant agents or adsorbent materials. Polyvinyl Pyrrolidone (PVP) 0.2 gL^{-1}. added to the culture medium, also activated charcoal at $1gL^{1}$ was used. Agar was added to the culture medium at $7gL^{-1}$ as gelling materials.

Multiplication Stage

The effect of several concentrations and combinations of cytockinin and auxin (ba & naa) on the multiplication of shoots after establishment of aseptic cultures was

studied. the explants were inoculated on a medium supplied with many concentrations of ba (0.0, 2.0, 4.0 and 6.0 mgl^{-1}) and naa at (0.0, 0.5, 1.0 and 2.0 mgl^{-1}). full strength culture media of ms (murashige and skooge,1962) and wpm (lloyd and mccown, 1980) with the same pgrs were compared during subcultures and 3 explants with 5 replicates were cultured in each jar. after six weeks of culture, the shoots per explants and node number were recorded.

Rooting

Shoots *in vitro* produced more than 2 cm long from apical buds explants were harvested for rooting. WPM (¼ strength) containing different concentrations of IBA (0.0, 0.1, 0.3, 0.5 mgL^{-1}), NAA (0.0, 0.1, 0.3, 0.5 mgL^{-1}). After 12 weeks of culture, root number, and mean root length were recorded.

Acclimatization

Following 12 weeks of rooting, agar was removed from the roots with distilled water and the plantlets were put in pots filled with a mixture of autoclaved peat moss and loam in a ratio (1:0.5) (V: V) covered by polyethylene to save humidity high relatively. The potted plants remained in the incubation room; survival rate was recorded after 30 days.

Genetic Stability

After 2 years of subculture, genetic stability was decided, the genome (DNA) extraction was done by using cetyltrimethylammonium bromide (CTAB) method (Weigand *et al.*,1993) from young leaves of mother and regenerated plants. For SRAP analysis, 24 primers were used for the genetic analysis of magnolia.

Table (1): A list of the locus name & sequences for each primer used in this research.

Forward primer 5 → 3	Sequence
Me 8	TGAGTCCAAACCGGTGC
Me 16	TGAGTCCAAACCGGGAC
Me 10	TGAGTCCTTTCCGGTCC
Me 17	TGAGTCCAAACCGGTAA
Me 14	TGAGTCCAAACCGGTGT
Me 22	TGAGTCCAAACCGGCTA
Reverse primers 3 → 5	Sequence
Em 14	TACGACGAATCCGGATG
Em 16	GACTGCGTACGAATTGCT
Em 15	GACTGCGTACGAATTCTG
Em 10	GACTGCGTACGAATTCAG
Em 8	GACTGCGTACGAATTGAG
Em 29	CGTAGCGCGTCAATTATG
Em 13	GACTGCGTACGAATTGGT
Em 22	GACTGCGTACGAATTCTC

DNA amplification for SRAP marker performed in the volume of 20 µl, including template DNA 2 µl, 10 µl of master mix, D.D.W 5 µl, ME 1.5 µl and EM 1.5 µl. Under the following conditions, PCR amplification protocol for SRAP was

performed: initial DNA denaturation for 5 minutes at 94˚C, denaturation for 60 seconds at 94 ˚C, annealing for 60 seconds at 35˚C, extension for 90 seconds at 72 ˚C for 5 cycles; denaturation at 94 ˚C for 60 seconds, annealing for 60 seconds at 50˚C, extension at 72 ˚C for 90 seconds for 35 cycles, extension for 10 minutes at 72 ˚C. Reactions of amplification were prepared using SRAP, and the product was run on 1% agarose gel and photographed under ultraviolet illumination.

Statistical Analysis

A completely randomized design (CRD) was used for this experiment and data were analyzed using ANOVA (variance of analysis). A significant difference among means using Duncan's multiple range test at level 5% was calculated, and results were represented as mean as ± standard error. All statistical analysis was performed using the computerized program of SAS (SAS 2010).

RESULTS AND DISCUSSION

Explants Sterilization

In this study, sterilization efficiency was high, (about 95%) when 70% (v/v) ethanol was used for 2 minutes, explant then soaked in 50% (v/v) of NaOCl for 20 minutes, and the contamination rate was 5%. Explants sterilization is necessary to establish plant tissue culture and the effective disinfection treatment should not damage the explant availability (Kang *et al.*,2020). This study indicated that 70 % ethanol for 2 min and NaOCl 50% (v:v) for 20 minutes was the most suitable treatment for sterilization of *Magnolia grandiflora* explants. NaOCl was used by many researchers to sterilize the explants (AL – Mallah, 2006).

Shoot Initiation

During the culture period, explants were cultured on an MS medium containing several concentrations of BA and NAA alone and with combinations at the initiation stage (Table 2). After four weeks of culture, shoots and nodes number were recorded, medium supplemented with BA at 1.0 mgL^{-1} + NAA at 0.05 mgL^{-1} was an optimal treatment at initiation stage, producing the higher number (2.60) of shoots/ explant, while BA at 0.5 mgL^{-1} alone, producing the low numbers (1.40) of shoots/ explants.

Table (2): The effect of BA, NAA and their combinations on number of shoots at the initiation stage of *Magnolia grandiflora* apical buds cultured on MS medium after four weeks.

BA (mgL^{-1})	NAA (mgL^{-1})				Mean effect of BA
	0.0	0.05	0.1	0.5	
0.0	0.40 f	1.80 a-e	2.00 a-d	2.40 ab	1.65 ab
0.5	1.20 d-f	1.40 c-e	1.00 ef	2.00 a-d	1.40 b
1.0	1.80 a-e	2.60 a	1.20 d-f	1.60 b-e	1.80 ab
2.0	1.60 b-e	2.20 a-c	1.80 a-e	2.40 ab	2.00 a
Mean effect of NAA	1.25 b	2.00 a	1.50 b	2.10 a	

This means following the same letters with a column, not showing significant difference from each other according to Duncan's multiple range test at a 5% level.

Prescience of BA, NAA alone or in combination was necessary for explant establishment and initiation. The interaction effect of auxins with cytokinins in growth increasing was clarified that the role of cytokinins was enhanced by the existence of auxins (Razdan, 2002) who mentioned that the activity of Cytokinin increased by the presence of auxins, producing a high shoot growth (Ibrahim *et al*., 2013; Ibrahim *et al*., 2020).

Table (3) illustrates an effect of BA, NAA, and combination on nodes number at initiation of *Magnolia grandiflora* shoot tip explant cultured on MS medium after four weeks. The highest number (2.60) of nodes/explants produced by the interaction between BA at 2.0 mgL^{-1} + NAA at 0.5 mgL^{-1}, while BA alone at 0.5 mgL^{-1} and NAA alone at 0.05mgL^{-1} produced the lowest number of nodes. Generally, auxin and cytokinin are considered the most important growth regulators in a micropropagation (George *et al.,* 2008; Kang *et al.,* 2020). Auxin and cytokinin balance are necessary for bud and root induction (Gasper *et al*.,1996; Abbas *et al.*,2014). BA increasing the number of shoots according to (Kasab Bashi and AL-Nooh, 2019). Medium supplemented with available growth regulators, the plant can induce cell division, differentiation of organs, morphogenesis, and the developments (Kang *et al*.,2020).

Table (3): The effect of BA, NAA and their combinations on the number of nodes at the initiation stage of *Magnolia* apical buds cultured on MS medium after four weeks.

BA (mgL^{-1})	NAA (mgL^{-1})				Mean effect of BA
	0.0	0.05	0.1	0.5	
0.0	0.40 c	0.60 c	1.60	1.00 a-c	0.90 b
0.5	1.20 a-c	1.00 a-c	0.80 bc	1.60 a-c	1.15 b
1.0	1.40 a-c	1.40 a-c	1.40 a-c	1.40 a-c	1.40 ab
2.0	1.40 a-c	1.40 a-c	2.40 ab	2.60 a	1.95 a
Mean effect of NAA	1.10 a	1.10 a	1.55 a	1.65 a	

This means following the same letters with a column, not showing significant difference from each other according to Duncan's multiple range test at a 5% level.

Shoot Proliferation

In the multiplication stage, MS & WPM media were compared to optimize growth with various concentration of BA, NAA and their combinations. An optimal concentration of BA was 6.0 mgL^{-1} and for NAA was 2.0 mgL^{-1} for shoot producing. Highs number of shoots/ explant (3.00) was achieved on MS medium provided with BA at 6.0 mgL^{-1} + NAA at 1.0 mgL^{-1}. Table (4). The combination of BA with NAA in culturing media enhanced significantly the shoot proliferation (Duhoky, 2010; Perveen *et al.,* 2011) The different species have different culture requirements, the most widely medium using with Magnoliaceae plant is MS medium (Luo *et al.*,2023). These results agree with many Magnoliaceae members that gave a maximum rate of multiplication under different concentrations, the variation may be due to a highly varying requires for every type of growth regulators depends on the cultural condition and cultured plants (Kim *et al*.,2020).

Table (5) reveals the effect of media, BA, NAA, and combinations on the node number at the multiplication of *Magnolia* apical bud explants. BA alone at 4.0 mgL^{-1} gave the best number (1.41) of nods as compared with other BA concentrations.

Table (4): The effects of media, BA, NAA & their combinations on the number of shoots at multiplication stage of *Magnolia* apical bud explants after 6 weeks.

media	BA	NAA				media*BA	Mean effect of media
		0.0	0.5	1.0	2.0		
MS	0.0	0.250 g	1.500 c-f	1.750 b-f	3.000 a	1.625 ab	1.766 a
	2.0	1.250 d-f	1.500 c-f	1.250 d-f	1.750 b-f	1.438 bc	
	4.0	1.750 b-f	2.250 a-d	2.000 a-e	1.750 b-f	1.938 a	
	6.0	1.750 b-f	2.000 a-e	3.000 a	1.500 c-f	2.063 a	
WP	0.0	0.750 fg	2.000 a-e	2.500 a-c	2.750 ab	2.000 a	1.719 a
	2.0	0.750 fg	1.250 d-f	1.000 e-g	1.500 c-f	1.125 c	
	4.0	1.500 c-f	1.250 d-f	2.250 a-d	2.250 a-d	1.813 ab	
	6.0	1.750 b-f	1.750 b-f	2.000 a-e	2.250 a-d	1.938 a	
Media*NAA	MS	1.250 c	1.813 ab	2.000 ab	2.000 ab	Mean effect of BA	
	WP	1.188 c	1.563 bc	1.938 ab	2.188 a		
BA*NAA	0.0	0.500 g	1.750 c-e	2.125 bc	2.875 a	1.813 a	
	2.0	1.000 fg	1.375 d-f	1.125 ef	1.625 c-f	1.281 b	
	4.0	1.625 c-f	1.750 c-e	2.125 bc	2.000 b-d	1.875 a	
	6.0	1.750 c-e	1.875 b-d	2.500 ab	1.875 b-d	2.000 a	
Mean effect of NAA		1.219 c	1.688 b	1.969 ab	2.094 a		

This means following the same letters with a column, not showing significant difference from each other according to Duncan's multiple range test at a 5% level.

Table (5): The effects of media, BA, NAA and their combinations on the number of nodes at the multiplication stage of *Magnolia* apical bud explants after 6 weeks.

Media	BA	NAA				BA Media	Mean effect of media
		0.0	0.5	1.0	2.0		
MS	0.0	0.00 f	1.50 a-d	2.25 a	1.00 b-f	1.19 bc	1.32 a
	2.0	0.50 d-f	0.75 c-f	0.75 c-f	2.13 a	1.03 c	
	4.0	1.25 a-e	1.25 a-d	1.50 a-d	1.00 b-f	1.25 bc	
	6.0	2.25 a	1.75 a-c	1.00 b-f	2.25 a	1.81 a	
WPM	0.0	0.25 ef	1.25 a-e	1.25 a-d	1.00 b-f	0.94 c	1.20 a
	2.0	1.75 a-c	1.00 b-f	0.75 c-f	2.00 ab	1.38 a-c	
	4.0	2.00 ab	1.75 a-c	2.00 ab	0.50 d-f	1.56 ab	
	6.0	0.75 c-f	1.00 b-f	1.00 b-f	1.00 b-f	0.94 c	
Media* NAA	MS	1.00 b	1.31 ab	1.38 ab	1.59 a	Mean effect of BA	
	WPM	1.19 ab	1.25 ab	1.25 ab	1.13 ab		
BA*NAA	0.0	0.13 e	1.38 a-d	1.75 ab	1.00 b-d	1.06 b	
	2.0	1.13 b-d	0.88 cd	0.75 de	2.06 a	1.20 ab	
	4.0	1.63 ab	1.50 a-c	1.75 ab	0.75 de	1.41 a	
	6.0	1.50 a-c	1.38 a-d	1.00 b-d	1.63 ab	1.38 ab	
Mean effect of NAA		1.09 a	1.28 a	1.31 a	1.36 a		

This means following the same letters with a column, not showing significant difference from each other according to Duncan's multiple range test at a 5% level.

NAA at 2.0 mgL^{-1} gave (1.36) nodes/explant but did not differ significantly from other NAA concentrations. The highest number (2.06) of nodes was obtained when the medium contained BA at 2.0 mgL^{-1} + NAA at 2.0 mgL^{-1}. However, there were no significant differences between the two types of media in the number of nodes and shoots of *Magnolia grandiflora* L.

Basal medium is the source of nutrients for *in vitro* plants and has an important function in plant tissue culture technique. Different types have different cultural requirements. Among the two basal media tested in this study (MS & WPM), there were no significant differences in the growth and proliferation of buds. Similar results were found by (Cui *et al.*, 2019) when they mentioned that there was no significant difference in multiplication and shoot number between MS, ½ MS, WPM, and DCR media on multiplication rates of *Magnolia sirindhorniae Noot*. & Chalermglin.

Rotting

Microshoots of approximately 2 cm long were used for rooting experiments. Several concentrations of NAA and IBA with different salt strengths of WPM were used to promote root formation in microshoots. The mean number of roots and root length were significantly affected by auxin type and salt concentrations. IBA was more active in root induction than NAA. Figure (1 and 2) indicated that WPM at fourth-strength (¼ x) with IBA at 0.5 mgL^{-1} produced the highest roots number and roots length when compared with other IBA concentration which has no root formation. (¼ x) WPM supplemented with the same concentrations of NAA not produced any roots. Plants belonging to Magnoliaceae have poor root formation (Kang *et al.*,2020). The number of roots and length of roots length were significantly affected by auxin type and salt concentrations. IBA at 0.5 mgL^{-1} gives the highest number of roots with the highest root length when compared with other IBA concentration which has no root formation. IBA produced 95% rooting of *Gardenia jasminoides* VAR. (Kassab Bashi, 2013).

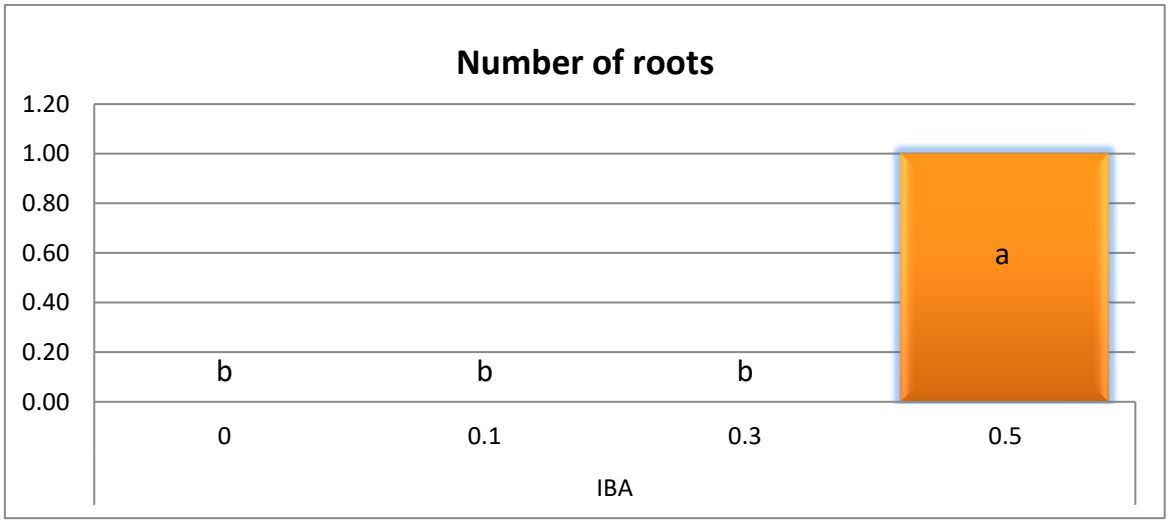

Figure (1): Effects of IBA concentration on the number of roots of *Magnolia grandiflora* L. microshoots cultured on WPM (¼) salt strength after 12 weeks.

(El-Gedawey *et al.*,2015) also mentioned that high levels of IBA show the best results in microshoot rooting of *Magnolia grandiflora*, this may be because of the reality that IBA usually takes a role in callus or root formation. *In vitro* roots of

magnolia grandiflora are treating the major problem in the development of microculture (Gercheva *et al.*, 2011) which is considered a deterrent *in vitro* system for this genus (Shi *et al.*, 2021) Luo *et al*, (2023) reported that raising the IBA concentration from 0 to 1.0 mgL^{-1} improved significantly the rooting percentage of *Manglietiastrum sinicum,* while medium containing NAA alone supports a lower percentage of rooting. These results are in agreement with those found by Bader *et al*. (2000).

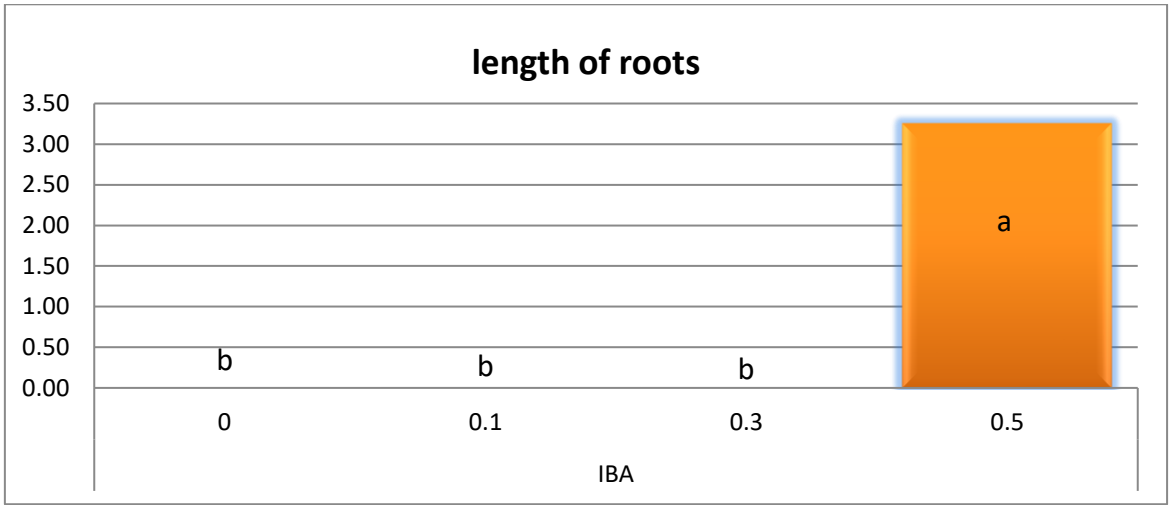

Figure (2): Effects of IBA concentration on the root length of *Magnolia grandiflora* L. microshoots cultured on WPM (¼) salt strength after 12 weeks.

Acclimatization

Transfer of plantlets from culture vessels to the soil is considered the most important steps in the micropropagation technique for every plant species. The plantlets were put in boxes with a mixture of autoclaved peat moss and loam in a ratio (1:0.5) (v: v). Then, covered with polyethylene to maintain high relative humidity. The survival rates of *Magnolia* plantlets during this study were reached to 80-85 %. These results agree with (Cui *et al.*,2019) who recorded the survival rate of 90.2% of *Magnolia sirindhorniae* plantlets. The survival rate of *Magnolia* 'Vulcan' was 87.5% (Kim *et al.*,2020). Plants formed in *in vitro* conditions grow under controlled conditions and when taken directly to a natural environment, they easily dehydrate and die, which is why it is necessary to carry out conditioning called hardening or acclimatization (Torres *et al.*, 2022). Figure (3).

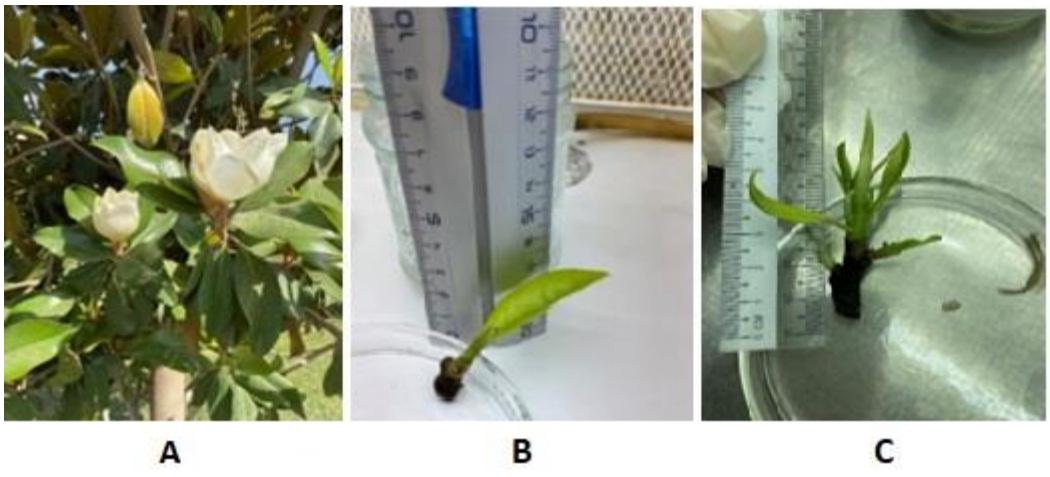

Figure (3): in *vitro* propagation of *Magnolia grandiflora* shoots tip explant, (A): Mather plant; (B) cotrol; (C) shoot initiation, BA 2.0 mg/l + NAA 0.05 mg/l; (D) bud proliferation on WPM containing BA at 6.0 mg/l + NAA at 2.0 mg/l; (E) Rooting on ¼ strength WPM at 0.5 mg/l IBA; (F) acclimatized plant.

Genetic Stability

SRAP Analysis

The genetic stability was assessed using an SRAP marker. In this study a total of 40 primer combinations (Em & Me) were used, but only 24 combinations were generated and produced a total of 310 bands scorable ranging between 100 to 3000 bp. The result of all these tested primers was monomorphic, between the mother plant and micro propagated plants, leading that there is no mutation and there being genetic stability using this protocol for micropropagation. Figure (4-8).

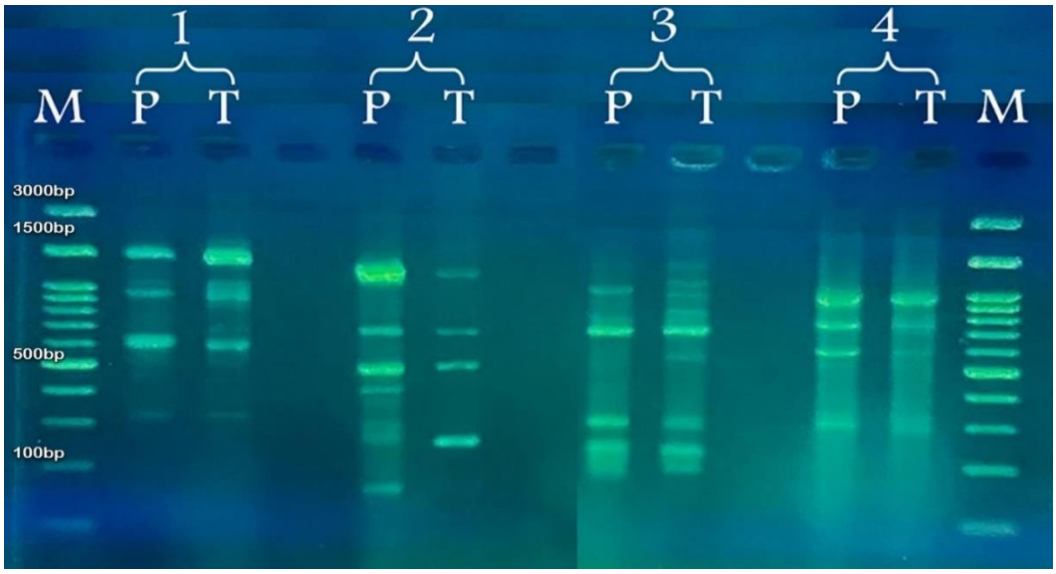

Figure (4): PCR product of *Magnolia grandiflora* obtained by ultraviolet light, the interactions between forward primer (Em) and reverse primers (Me). (M): Marker. (P): Parent. (T): Tissue culture produced plant.

1) Em 14 + Me 8
2) EM 16 + Me 16
3) Em 10 + Me 10
4) Em 8 + Me 8

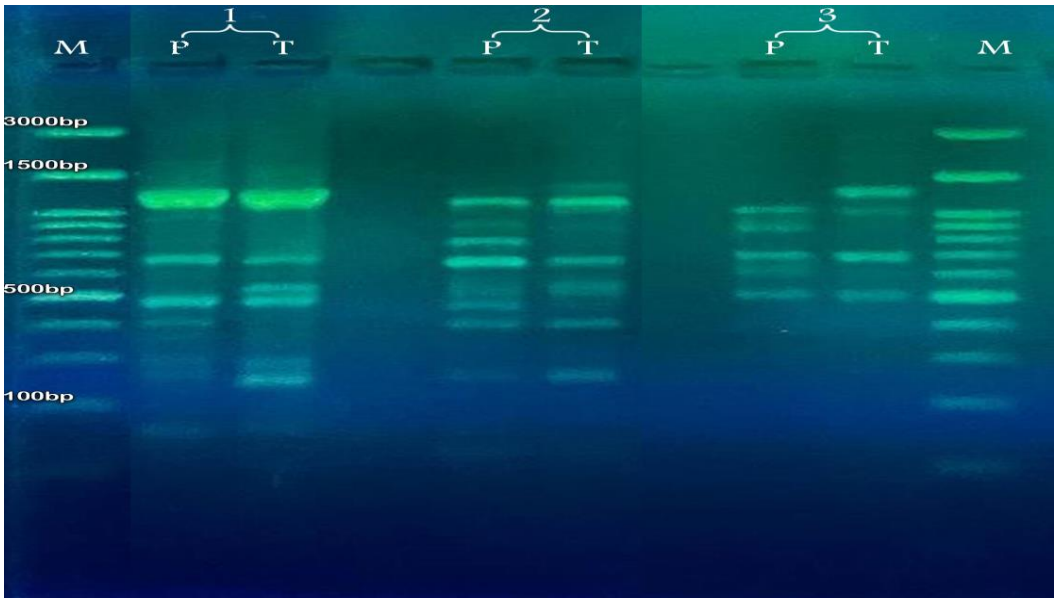

Figure (5): PCR products of *Magnolia grandiflora* obtained with ultraviolet light, reaction between the forward primers (Em) and reverse primers (Me). (M): Marker. (P): parents. (T): tissue culture produced plant.

1) Em 16 + Me 17
2) Em 14 + Me 16
3) Em 15 + Me 10

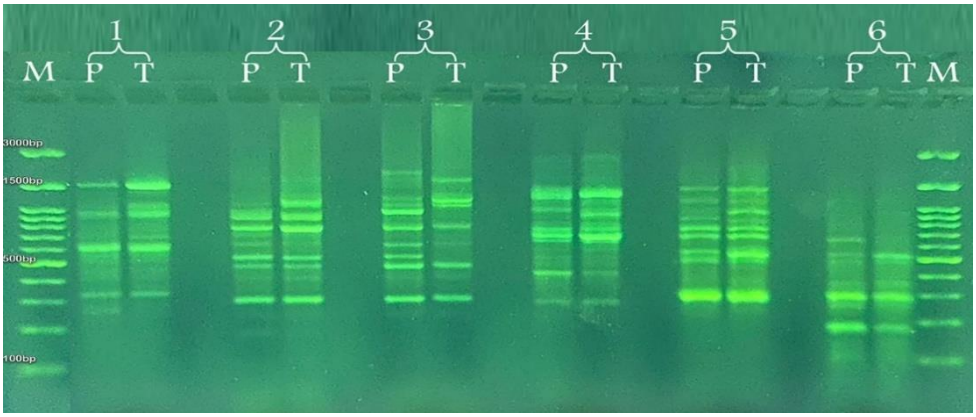

Figure (6): PCR products of *Magnolia grandiflora* gave with ultraviolet light, reaction between the forward primer (Em) and reverse primers (Me). (M): marker. (P): parent. (T): tissue culture plant.

1) Em 14 + Me 8
2) Em 16 + Me 10
3) Em 16 + Me 8
4) Em 15 + Me 8
5) Em 10 + Me 8
6) Em 8 + Me 16

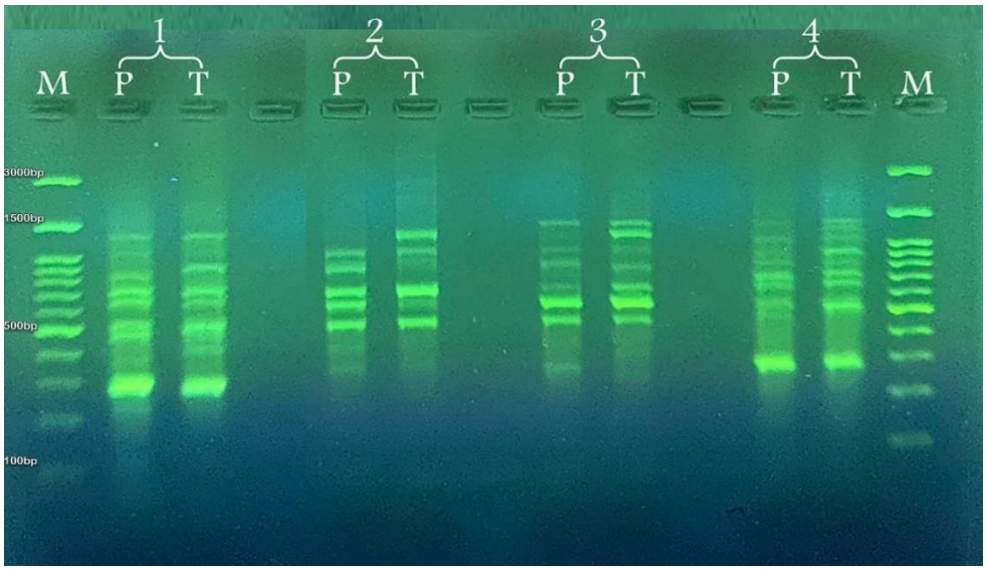

Figure (7): PCR products of *Magnolia grandiflora* L obtained by ultraviolet light, the reaction between forward primers (Em) and reverse primer (Me). (M): marker. (P): parent. (T): tissue culture plant.

1) Em 8 + Me 10
2) Em 15 + Me 17
3) Em 14 + Me 17
4) Em 8 + Me 14

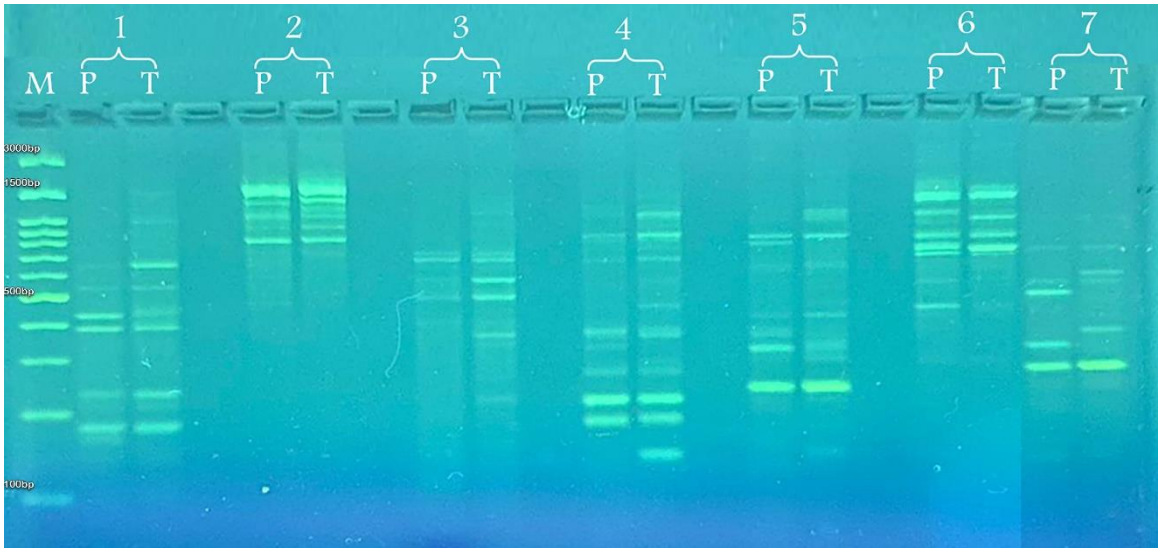

Figure (8): PCR products of *Magnolia grandiflora* L obtained with ultraviolet light, reaction between the forward primer (Em) and reverse primers (Me). (M): Marker. (P): Parent. (T): Tissue culture produced plant.

1) Em 13 + Me 10
2) Em 13 + Me 8
3) Em 13 + Me 16
4) Em 22 + Me 22
5) Em 22 + Me 10
6) Em 22 + Me 18
7) Em 29 + Me 17

The stability of plants during tissue culture may affected by many factors (Kang *et al.*,2020). Though, clonal materials can be achieved by *in vitro* culture, the composition of the medium, using plant growth regulators and stress during micropropagation may cause mutation and induce a genetic variation in regenerated plants (Ioanuidis *et al.*,2022 and Luo *et al*.,2023). So, it's important to estimate a genetic uniform for regenerated plants. For this study, the SRAP marker was used to analyze the genetic stabilization of regenerated plants after 2 years of subcultures. The products of amplification show monomorphic for micropropagated and mother plant, statement the genetic stability of micropropagated plants. Results are consistent with previous studies results (Cui *et al*.,2019; Kang *et al.*,2020 and Tomiezak *et al*., 2023), which suggested that the proliferation of buds could minimize the chance of genetic instability.

CONCLUSIONS

A reliable and efficient regeneration protocol for micropropagation of Magnolia grandiflora was established. MS supplied with BA at 2.0 mgL^{-1} + NAA at 0.5 mgL^{-1} was the optimal medium for bud induction. MS medium with BA at 6.0 mgL^{-1} and NAA at 1.0 mgL^{-1} was the best for shoot proliferation. There were no significant differences between MS and WPM in terms of multiplication. IBA was more suitable for root initiation than NAA. They were using DNA markers (SRAP markers) as a sufficient method to detect the stability of micropropagated plants.

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CONFLICT OF INTEREST

The researchers declare that they do not have any competing tests and there is no conflict of interest.

استخدام علامة SRAP للكشف عن الثبات الوراثي لنبات *Magnolia grandiflora* L. الناتجة بالإكثار الدقيق

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الخلاصة

نفذت هذه الدراسة في كل من مختبري زراعة الأنسجة النباتية ومختبر المولیكیولر بایولوجي مركز أبحاث كلية العلوم في جامعة دهوك خلال فترة أب 2021 ولغاية شهر أيلول 2023. استخدمت البراعم الطرفية كجزء نباتي خلال التجربة. أظهرت نتائج التعقيم بأن استخدام كحول الأيثانول بتركيز (70% حجم/حجم) لمدة 2 دقيقة ثم غمر الجزء النباتي في محلول الكلوركس NaOCL بتركيز 2.5% لمدة 20 دقيقة كانت أفضل

معاملة للتعقيم. خلال مرحلة النشؤ كانت وسط ألزراعة MS أحسن وسط للزراعة حيث أعطت أكبر عدد (2.60) من الفروع عندما تم تجهيزه بكل من BA بتركيز 1ملغم/لتر وNAA بتركيز 0.05 ملغم/لتر كما أعطت أكبر عدد من البراعم (2.60) بإضافة كل من BA بتركيز 2ملغم/لتر وNAA بتركيز 0.5 ملغم/لتر. في مرحلة التضاعف تم الحصول على أكبر عدد من الأفرع (2.50) بإضافة كل من BA بتركيز 6 ملغم/لتر وNAA بتركيز 1ملغم /لتركما أنتجت أكبر عدد من البراعم (2.06) عند تجهيز الوسط بكل من BA بتركيز 2ملغم/لتر وNAA بتركيز 2ملغم/ لتر ولم يكن هنالك فروقات معنوية بالنسبة لوسط التضاعف MS وWPM على معدل التضاعف. تطور نمو الجذور في وسط WPM ربع القوة بإضافة IBA بتركيز 0.5 ملغم/لتر. في مرحلة الأقلمة تم زراعة النباتات في مزيج تتكون من البيتموس واللوم بنسبة حجمية (0.5:1) حيث بلغت نسبة ألنجاح 80–85%. تم استخدام 24 بادئ مركب من SRAP لتحديد الثبات الجيني للنباتات المتجددة وأظهرت نتائج التحليل بأن هنالك استقرار جيني لنباتات المتجددة عند مقارنتها مع نبات الأم.

الكلمات المفتاحية: زراعة الأنسجة النباتية، مغنوليا، ألأقلمة، DNA ماركر.

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