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Horticulture

An efficient protocol for *in vitro* propagation of purple-leaf plum (*Prunus cerasifera*)

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Abstract

The present study aimed to establish an efficient *in vitro* propagation protocol for purple-leaf plum (*Prunus cerasifera* Ehrh). Clorox at 15% (v/v) or $HgCl_2$ at 0.1 % (w/v) for 10 min each gave the best results for both decontamination (100 and 90.2) and survival (90 and 92) percentages, respectively. The effect of three cytokinin types (BAP, Kin and 2ip) at four concentrations (0.5, 1.0, 1.5 and 2.0) mg/L were studied during multiplication stage in comparing with hormonal-free medium. Applying BAP at 1.5 mg/L induced the initiated buds to develop into shoots (100% shooting) during the three successive subcultures. Adding BAP at 1.5 or 2.0 mg/L gave the highest significant shootlets multiplication rate (2.13 and 2.15 shootlet/ explant, respectively). While the longest shootlets (1.92 cm) were recorded on MS medium augmented with 1.5 mg/L of 2ip. Adding 0.4 mg/L of IBA to the culture medium lead to 100% rooting of shootlets. Using peat moss + perlite (1:1 v/v) as a putting medium for *ex vitro* acclimatization of plantlets resulted in the best results for survival, height, stem fresh and dry weights of acclimatized plantlets. The amplified DNA fragments using 844-A (for ISSR) and A2 (for RAPD) primers to compare between donor and micropropagated plants. Data showed that the maximum number of bands (11 and 9), respectively of DNA fragments with molecular size ranging between 226.98- 1007.92 and 215.16- 1619.62 bp. Data indicated that micropropagated plants and donor plant are much closed to each other and the micropropagation protocol genetically safe.

Key words: *Prunus cerasifera*, *in vitro* propagation, acclimatization

Introduction

Purple-leaf plum (*Prunus cerasifera* Ehrh) is a small shrubby tree belongs to family Rosaceae. It is commonly cultured for landscape as a decorative tree because of its colourful leaves and numerous and exquisite pale pink flowers decorating the branches throughout spring (Szekely and Dagmar, 2011). According to Hedrick (1911) it was used early in breeding programs.

Plant tissue culture has provided a great contribution to the micropropagation and considered as a potent utensil for mass production of healthy and reliable of ornamental, forest trees and fruit tree rootstocks in recent decades (Aghaye *et al.*, 2013). It is providing a huge number of superior clonal seedlings in a short time all over the year (Cui *et al.,* 2019). The success of tissue culture protocol depends on surface sterilization of explants (Dodds and Roberts, 1985). Murashige and Skoog (MS) is considered the most common used culture medium because of it contains high levels of macro and some of the micronutrients,

particularly boron and manganese (Cohen, 1995). Growth regulators are one of the most important factors that used to control *in vitro* growth and development of plants according to their types and concentrations. Cytokinins have a major role on the evolution of plant, i.e. regulation of shoot formation, propagation and the induction of cell division and expansion (Mok and Mok, 2001). Acclimatization stage is the final stage of the micropropagation operation after in which the produced plantlets can transferred to soil and should be considered as serious to get benefits of tissue culture (Chandra *et al.,* 2010).

Our study aimed to optimize a protocol for *in vitro* production true to type plantlets of purple-leaf plum (*Prunus cerasifera*) species of great importance for landscape and horticultural breeding as it imported from abroad.

Materials and Methods

This study was carried out in tissue culture and germplasm conservation laboratory, Horticulture Research Institute, Agricultural Research Centr during 2018 and 2019 aimed to establish a protocol for *in vitro* production of true to type plantlets of *Prunus cerasifera* through direct regeneration.

Plant material and explant disinfection

Stem nodal explants were collected from a privet farm of My Garden, Alex. Desert Road. The explants were cut into approximately 1cm micro-cutting pieces, pre-sterilized successfully in soapy water for 30 min then washed with running tap water for 20 min. To optimize a protocol for surface sterilization, explants were surface disinfected as follows: immersion in ethanol (70% v/v) for 60 sec., and washed one time with sterile distilled water, then dipping for 15 min in Clorox (5.25% NaOCl) at 7, 10 or 15 % (v/v) or mercuric chloride at 0.05, 0.1or 0.15 % (w/v) with drops of tween 20, another treatment of surface sterilization was applied; 10% Clorox for 10 min followed by 0.05 % mercuric chloride for 5 min. Finally, the sterilized explants were washed with sterilized distilled water three times to get rid of the residue of disinfectants. After removing the basal ends, three explants were implanted vertically in 200 ml glass jars containing 30 mL of full strength Murashige and Skoog, (1962) medium with 3% sucrose, and 7.0 g L^{-1} of agar; unless otherwise stated used for viability and contamination studies.

Incubation conditions

Cultures were grown at 24 ± 2 °C under 16 hours (3000 lux) photoperiod using white fluorescent lamps controlled automatically.

Multiplication stage

To optimize cytokinin type and concentration, three types (BAP, Kin or 2ip) with four concentrations (0.5, 1.0, 1.5 and 2.0 mg/L) comparing with hormone-free medium were applied through three successive subcultures with four weeks intervals. Shoot formation percentage (shooting capacity), the number of shootlets/explant, shootlet length (cm) and number of leaves formed per each shootlet were recorded at the end of each subculture.

Rooting stage

Two experiments were conducted to study rooting behaviors of the grown shootlets in different treatments:

A: Effect of the strength of hormone-free MS-medium (¼, ½, ¾ and full strength).

B: Effect of IBA concentration (full strength MS medium with 0.0, 0.2 and 0.4 mg/L). To achieve this trail, homogeneous micro shootlets produced from multiplication stage were cultured individually and incubated for 6 weeks. Rooting percentage, number of the initiated roots/ shootlet and length of the formed roots were estimated.

***Ex vitro* acclimatization stage**

Well rooted plantlets (6-8 cm) were washed to erase intact medium and implanted into 200 ml plastic pots containing potting media of peat moss, peat moss + sand, peat moss + vermculite (1:1 v/v) which saturation with 0.1% Topsin-M70 (Thiophanate-methyl) fungicide. Culture vesicles were covered with transparent polyethylene bags and maintained in fiber-glass-house. Two weeks later, one pore per the polyethylene bag was performed, and after another two weeks the bags were gradually erased. The acclimatized plantlets were irrigated twice a week for four weeks. Survival percentage, plantlet height and root length were recorded at the end of this stage.

DNA fingerprint

Inter Sequence Short Repeat (ISSR(

The thermocycler was programmed as follows: denaturation at 94 °C for 5 min (one cycle), then for 1 min (35cycles), annea1ing for min at 45°C, and extension for 1 min at 72 °C followed by a final extension step at 72°C for 5 min (Kumar *et al*., 2010 (.

Random Amplification Polymorphism DNA-PCR (RAPD)

The thermocycler was programmed as follows: denaturation one at 94°C for 4 min (one cycle), 94°C for 1 min (45 cycles), annea1ing for 1 min at 36°C and extension for 2 min at 72°C (one cycle) followed by a final extension step at 72°C for 10 min (Rajapakse *et al*., 1995). PCR products were fractionated on agarose gel 2% (w/v) electrophoresis in 0.5x TBE buffer. The band size was estimated by using DNA molecular weight marker using gel documentation system.

No	ISSR		RAPD	
	Primer	Sequences **5`----3`**	Primer	Sequences **5`----3`**
1	17898 A	CAC ACA CAC ACA AC	A2	TGCCGAGCTG
2	UBC 807	AGA GAG AGA GAG AGA GT	C5	GATGACCGCC
3	UBC 809	AGA GAG AGA GAG AGA GG	C11	AAAGCTGCGG
4	UBC 811	GAG AGA GAG AGA GAG AC	C19	GTTGCCAGCC
5	HB 15	GTG GTG GTG GC		
6	844 A	CTC TCT CTC TCT CTC TAC		

Experimental design and data statistical analysis

A completely randomized design experiment with one factor was used for explant disinfection, rooting and acclimatization stages. While, shoot multiplication stage trail was designed with two factors in a completely randomized design. ANOVA table was obtained and means were compared at 5% level using the Dancan`s multiple range test (Steel and Torrie, 1980).

Results and discussions

Surface sterilization

The present investigation was carried out to optimize a protocol for surface sterilization of *Prunus cerasifera* (Table 1). Using Clorox at 15% or $HgCl_2$ at 0.1 % for 10 min gave the highest results for both decontamination (100% and 90.2%) and survival (90.0 and 92.0%) percentages, respectively. At low concentrations of the used disinfectants (7% Clorox and 0.05% $HgCl_2$), the decontamination percentages were 35.5 and 57.0 %, respectively. Vujović *et al.* (2012) findings suggested that 12 min-immersion in 10% (v/v) commercial bleach solution (0.4%, w/v NaOCl) was effective sterilizing agent for prunus rootstocks. Also, Yildirim *et al.* (2011) suggested that raising Clorox concentration from 5% to 20% had inversely proportion with contamination percentage of apricot (*Prunus armeniaca*) axillary buds.

Table (1): Effect of Clorox and mercuric chloride on decontamination and survival percentages of *Prunus cerasifera* explants during establishment stage.

Parameters Treatments	Decontamination (%)	Survival (%)
Clorox 7 % for 10 min	35.5 e	100.0 a
Clorox 10 % for 10 min	85.7 c	100.0 a
Clorox 15 % for 10 min	100.0 a	90.0 b
$HgCl_2$ 0.05 % for 10 min	57.0 d	100.0 a
$HgCl_2$ 0.10 % for 10 min	90.2 b	92.0 b
$HgCl_2$ 0.15 % for 10 min	100.0 a	70.0 d
Clorox 10 % for 10 min + $HgCl_2$ 0.05 % for 10 min	100.0 a	75.0 c

Means having the same letter(s) in each column are not significantly differed from each other at 5% level according to Duncan`s multiple range.

Shoot multiplication stage

Shoot formation percentage

As in shown in Table (2), it is clear that the shoot formation was declined significantly with repeated subculturing. At the same time, shooting capacity was significantly affected by cytokinin types and concentration. Applying BAP at 1.5 mg/L in the culture medium resulted in inducing all buds to develop into shoots (100% shooting capacity) during the three successive subcultures. Whereas, using hormone-free MS medium (control) minimized the shoot formation to the least significant values (48 and 29%) during the second and third subculture, respectively. It seemed that repeated subculturing had insignificance effect on shooting ability of nodal explants that were grown on medium enriched with different concentrations of BAP or 2ip. While, the shooting ability of that cultured on medium augmented with kin was decreased significantly with repeated subculturing. In this respect, Lakshimi and Mythili (2003) stated that BAP enhances shoot multiplication of many plant species, which is in harmony with the results of the present study. Also, George (1993) mentioned that BAP break bud dormancy and induces shoot formation from lateral buds.

Number of shoorlets/explant

The formed Shootlet number was increased with repeated culture as shown in Table (2). The mean effect of cytokinins indicated that BAP at 1.5 or 2 mg/L had the powerful to induce shoot formation as compared 2ip and kin. In this concern, George *et al.* (2008) stated that BAP increases shoot formation and emergence lateral buds from dormancy. As for the interaction effect, number of formed shootlets showed a different behavior as a result of using different types and concentrations of cytokinins. Data showed that using 2ip at different concentrations and kin at 1.0, 1.5 and 2.0 mg/L had no significant effect on the number of shootlet/explant during the three successive subcultures. Whereas, number of the formed shootlets per explants cultured on MS medium amended with 0.5 mg/L kin insignificantly increased from first to the second subculture and significantly from the second to the third subculture. In the another trend, BAP took a different direction according to its concentration. When it was added at concentration of 0.5 mg/L, the number of shootlets was decreased insignificantly with repeated subculturing. The number of shootlets was decreased insignificantly from 1st to 2nd and significantly from 2nd to 3rd subculture when BAP was added at 1.0 mg/L to the culture medium. Augmenting MS medium with 1.5 mg/L BAP resulted in a significant increment in shootlet number/ explant from 1st to 2nd subculture and then significant reduction from 2nd to 3rd subculture. Using the highest level of BAP (2.0 mg/L) insignificantly minimized the shootlet number/ explant from the 1st to the 2nd subculture and significantly maximized it to reach the highest value (2.79 shootlets per explant) in the third subculture. According to the obtained results it could be concluded that cytokinins differed in their uptake, realization and mode of action. In the present experiment, cytokinins could be classified into two groups: highly effective group (BA), being more active, i.e. more multiplication rate, and weakly effective group (2iP and Kin) exhibited weak effects on multiplication rate. These results agreed with results obtained by Dj *et al*. (2008) on prunus and Zenna and Taha (2018) on *Antigonon leptopus*; they suggested that BAP displayed more noticeable effect than TDZ, 2ip and kinetin.

Length of shootlets

Repeating subculture had no significant effect on the length of shootlets (Table 2). While, cytokinins at various concentrations used in the present study had a significant effect on shootlet length. Using 2ip at concentrations over 0.5 mg/L resulted in cell expanding and therefor shootlet elongation. The highest significant shootlet length (1.92 cm) was recorded for the average of MS medium augmented with 1.0 mg/L 2ip. The interaction between subculturing and different concentrations of the various cytokinins showed different directions. Length of shootlets derived from explants cultured on MS medium fortified with 2ip at 1.5 or 2.0 mg/L or BAP at 1.0 mg/L was increased with repeating subculture. So, it could be suggested that to get long shootlets we have to use 2ip as a cytokinin at 1.5 or 2.0 mg/L in multiplication stage. The efficacy of BAP on bud induction and shoot multiplication was noticeable but, less effective on shoot elongation compared with Kin and 2ip that was supported by previous studies on *in vitro* propagation of *Chlorophytum arundinaceum* (Lattoo *et al.,* 2006) who reported that Kin. enhanced the *in vitro* shoot elongation.

Number of leaves per shootlet

As shown in Table (2), repeating subculture had no significant effect of the number of leaves formed per shootlet. Whereas, cytokinins had a significant effect. Augmentation of MS medium with 2ip at 1.0 or 2.0 mg/L improved leaves formation to record the highest significant values (10.53 and 10.55 leaves/ shootlet), respectively. It also noticed that the number of leaves per shootlet was increased with repeating subculture with the mentioned previous two treatments (MS with 1.0 or 2.0 mg/L 2ip). While, it took a different direction with the other cytokinin types (BAP and kin) at different concentrations. Similar results were observed on *Ficus carica* with the best number of leaves that was obtained with medium supplemented with 2.5 mg/L 2ip in comparison with BAP and kin (Mustafa and Taha, 2012). On the other hand, Villa *et al.,* (2005) demonstrated that blackberry plants with the highest leaf number (7.89) were achieved in the presence of 1.0 mg L^{-1} BAP.

Rooting stage

In vitro rooting is essential stage for development of micropropagated plants in the field. Rooting ability was significantly varied according to salt strength of MS medium (Table 3-a). Shootlets cultured on full MS or 1/4 strength of MS media recorded higher rooting percentages compared to those cultured on 1/2 or 3/4 MS strengths. Number of the formed roots per shootlet cultured on 1/4 or 1/2 strengths of MS macro and micro nutrients were higher than those formed per shootlets grown on 3/4 or full MS strengths.

Table (2): Effect of cytokinen types and concentration on shooting behaviors of *Prunus cerasifera* during multiplication stage

		Subculture			
	PGR (mgl^{-1})	**1st**	**2nd**	**3rd**	**Mean (B)**
Shoot formation percentage	Hormone-free	87.0 a-d	48.0 f	29.0 g	54.67 e
	BAP 0.5	88.0 a-d	80.0 c	84.0 b-d	84.00 d
	BAP 1.0	92.0 a-c	92.0 a-c	92.0 a-c	92.00 bc
	BAP 1.5	100.0 a	100.0 a	100.0 a	100.00 a
	BAP 2.0	100.0 a	100.0 a	92.0 a	97.33 ab
	Kin 0.5	96.0 ab	92.0 a-c	75.0 de	87.67 cd
	Kin 1.0	96.0 ab	84.0 b-c	71.0 e	83.67 d
	Kin 1.5	96.0 ab	88.0 a-d	82.0 c-e	88.67 cd
	Kin 2.0	96.0 ab	93.0 a-c	86.0 c-d	91.67 bc
	2ip 0.5	84.0 b-c	88.0 a-d	80.0 c-e	84.00 d
	2ip 1.0	96.0 ab	92.0 a-c	92.0 a-c	93.67 a-c
	2ip 1.5	100.0 a	96.0 ab	87.0 a-d	94.33 a-c
	2ip 2.0	92.0 a-c	100.0 a	100.0 a	97.33 ab
	Mean (A)	94.08 A	88.69 B	82.31 C	
Shootlet number per explant	Control	1.00 d	1.00 d	1.00 d	1.00 d
	BAP 0.5	1.30 d	1.06 d	1.00 d	1.12 d
	BAP 1.0	2.08 bc	1.98 c	1.11 d	1.72 b
	BAP 1.5	1.92 c	2.76 a	1.72 c	2.13 a
	BAP 2.0	1.88 c	1.78 c	2.79 a	2.15 a
	Kin 0.5	1.00 d	1.14 d	2.41 b	1.52 c
	Kin 1.0	1.04 d	1.00 d	1.20 d	1.08 d
	Kin 1.5	1.00 d	1.00 d	1.18 d	1.09 d
	Kin 2.0	1.00 d	1.00 d	1.00 d	1.00 d
	2ip 0.5	1.00 d	1.00 d	1.33 d	1.11 d
	2ip 1.0	1.00 d	1.00 d	1.00 d	1.00 d

	2ip 1.5	1.00 d	1.00 d	1.00 d	1.00 d
	2ip 2.0	1.00 d	1.00 d	1.00 d	1.00 d
	Mean	1.25 b	1.31 ab	1.36 a	
Shootlet length (cm)	Control	0.85 i-l	0.57 kl	0.42 l	0.63 f
	BAP 0.5	1.31 d-i	1.06 g-k	1.09 f-k	1.15 cd
	BAP 1.0	1.22 e-j	1.67 b-g	1.92 a-c	1.60 b
	BAP 1.5	1.16 f-k	1.27 d-j	1.22 e-j	1.22 cd
	BAP 2.0	1.24 d-j	1.11 f-k	0.98 h-l	1.11 de
	Kin 0.5	1.17 e-k	0.86 i-l	0.80 i-l	0.94 de
	Kin 1.0	0.97 h-l	0.97 h-l	1.09 f-k	1.01 de
	Kin 1.5	1.34 c-i	0.81 i-l	1.07 g-k	1.08 de
	Kin 2.0	1.05 g-l	0.65 j-l	0.67 j-l	0.79 ef
	2ip 0.5	1.07 g-l	1.19 e-k	1.55 c-h	1.27 cd
	2ip 1.0	1.37 c-i	1.83 b-d	1.11 f-k	1.44 ab
	2ip 1.5	1.53 c-h	1.78 b-e	2.44 a	1.92 a
	2ip 2.0	1.38 c-i	1.71 b-f	2.14 ab	1.74 ab
	Mean	1.21 a	1.19 a	1.27 a	
Number of leaves per shootlet	Control	6.00 jk	6.10 i-k	5.28 k	5.79 e
	BAP 0.5	8.28 f-h	7.54 h-j	7.84 gh	7.89 d
	BAP 1.0	8.46 f-h	9.16 e-h	9.42 d-g	9.01 bc
	BAP 1.5	9.18 e-h	9.10 e-h	9.44 d-g	9.24 b
	BAP 2.0	8.52 e-h	8.59 e-h	8.72 e-h	8.61 b-d
	Kin 0.5	9.06 e-h	7.70 g-i	7.55 h-j	8.11 cd
	Kin 1.0	7.56 h-j	8.40 f-h	8.66 e-h	8.21 cd
	Kin 1.5	9.14 e-h	9.28 e-h	9.74 c-f	9.39 b
	Kin 2.0	8.10 f-h	8.30 f-h	8.24 f-h	8.21 cd
	2ip 0.5	8.34 f-h	8.04 s-h	8.24 f-h	8.21 cd
	2ip 1.0	9.40 d-g	11.00 a-d	11.18 a-	10.53 a

			c	
2ip 1.5	10.28 d-e	9.08 f-h	9.24 e-h	9.53 b
2ip 2.0	7.98 f-h	11.46 ab	12.20 a	10.55 a
Mean	8.45 a	8.75 a	8.90 a	

Means having the same letter(s) in each column are not significantly differed from each other at 5% level according to Duncan`s multiple range

Previous studies suggested that the decrease of macro- and micro salts by half in the nutrient medium usually improves rhizogenesis of some plant species (Trejgell *et al*., 2009).Adding 0.4 mg/L IBA to MS culture medium significantly lead to 100% rooting of shootlets (Table 3-b). Values of the other studied parameters (number of roots per plants and length of roots) were reached the highest significant values (7.06 roots/plantlet and 4.38 cm, respectively) on the same treatment. The effect of IBA on micropropagated shootlets was investigated by Asghar *et al*., (2011) and they stated that the adding of auxins to *in vitro* propagated microshoots being increase number of the formed roots by activation the endogenous contents of enzymes. Also, Liu *et al*. (2002) demonstrated that auxin promote lateral root initiation through complicated process of repetitive cell division. George *et al*. (2008) reported that auxins are necessary for the keeping up of the plant polarity. Other study suggested that conversion IBA to IAA is followed by peroxisomal NO, which is essential for root initiation (Schlicht *et al*., 2013).

Table (3-a): Rooting response of *Prunus cerasifera* shootlets as affected by MS strength

	Rooting response		
Treatments	Rooting (%)	Root no./shootlet	Root length (cm)
Full MS	77.62 a	1.71 b	3.35 a
3/4 MS	21.60 c	1.24 b	1.62 b
1/2 MS	39.89 b	2.80 a	2.40 ab
1/4 MS	82.86 a	2.90 a	3.30 a

Means having the same letter(s) in each column are not significantly differed from each other at 5% level according to Duncan`s multiple range

Table (3-b): Rooting response of *Prunus cerasifera* shootlets as affected by IBA concentration

	Rooting response		
Treatments	Rooting (%)	Root no./shootlet	Root length (cm)
Free MS	77.62 b	1.71 b	3.35 a
MS + 0.2 mg/L IBA	73.90 b	3.20 b	4.32 a
MS + 0.4 mg/L IBA	100.00 a	7.06 a	4.38 a

Means having the same letter(s) in each column are not significantly differed from each other at 5% level according to Duncan`s multiple range

***Ex vitro* acclimatization**

The effect of three mixtures as potting media on the behaviors of the acclmatized plantlets was presented in Table 4. The best results for plantlet survival, plantlet height and stem fresh and dry weights of acclimatized plantlets were obtained from using peat moss + perlite (1:1). While, the longest roots were obtained for plantlets acclimatized in peat moss. Perlite is an important component in the potting mixture when mixed with peat moss. This response may be due to the ability of the mixture to provide enough moisture and aeration to the plants, thereby resulting in good growth (Gonbad *et al.,* 2013).

Table (4): Effect of potting media on growth characteristics of *Prunus cerasifera* micropropagated plantlets during acclimatization stage

	Growth parameters						
Potting media	Survival (%)	Plant height	Stem f.w (g/plant)	Stem d.w (g/plant)	Root length (cm)	Root f.w (g/plant)	Root d.w (g/plant)
P1	59.0 a	7.80 a	1.53 a	0.39 a	6.67 c	0.14 b	0.06
P2	37.0 b	6.20 b	1.34 a	0.29 b	7.77 b	0.13 b	0.06
P3	34.7 b	4.47 c	1.02 b	0.17 c	8.27 a	0.31 a	0.04

P1= peat moss + perlite (1:1), P2= Peat moss + sand (1:1), P3= Peat moss

Means having the same letter(s) in each column are not significantly differed from each other at 5% level according to Duncan`s multiple range

DNA fingerprint

Identification of ISSR primers

The six primers which generated reproducible and scorable polymorphic markers were selected for further analysis. They produced numerous band profiles containing amplified

DNA fragments ranging from 5 to 11, while the number of polymorphic fragments ranged from 1 to 6 (Table 5 and Fig. 2). A maximum number of 11 fragments was amplified with the primers (844-A) and a minimum number of 5 fragments was amplified with the primer (UBC809). The total number of reproducible fragments amplified by the six primers reached 45 bands, of which 6 were polymorphic fragments. This represented a level of polymorphism of 13%, which indicates a very high level of polymorphism among the mother and micro propagated studied genotype. The size of the amplified fragments also differed according to primers and ranged from 300 to 1700 bp (Fig. 2). The ISSR analysis revealed a high level of polymorphism among mother and micro propagated genotype, which enabled accurate analysis of the genetic distance. The genetic similarity among the mother and micro propagated of *Prunus cerassifera* was estimated based on the scored ISSR data matrix, showed that the genetic similarity was 86.8% which reflects a high level of similarity at their DNA level as expected between the genotype and it self with treatment

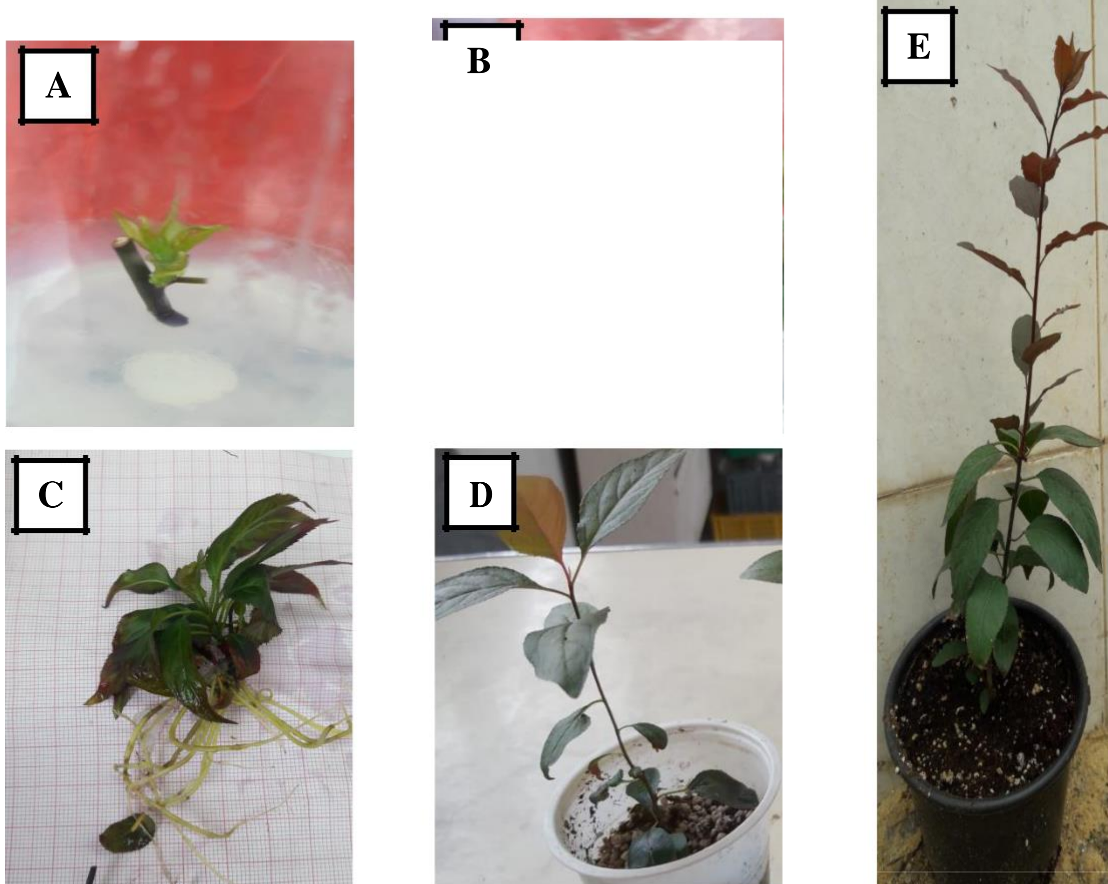

Fig. (1): Micropropagation stages, A: bud emergence during establishment stage, B: multiplication stage, C: well rooted plantlets after rooting stage, D: 6 weeks old acclimatized plantlets and E: 3 months old grown seedlings after acclimatization stage

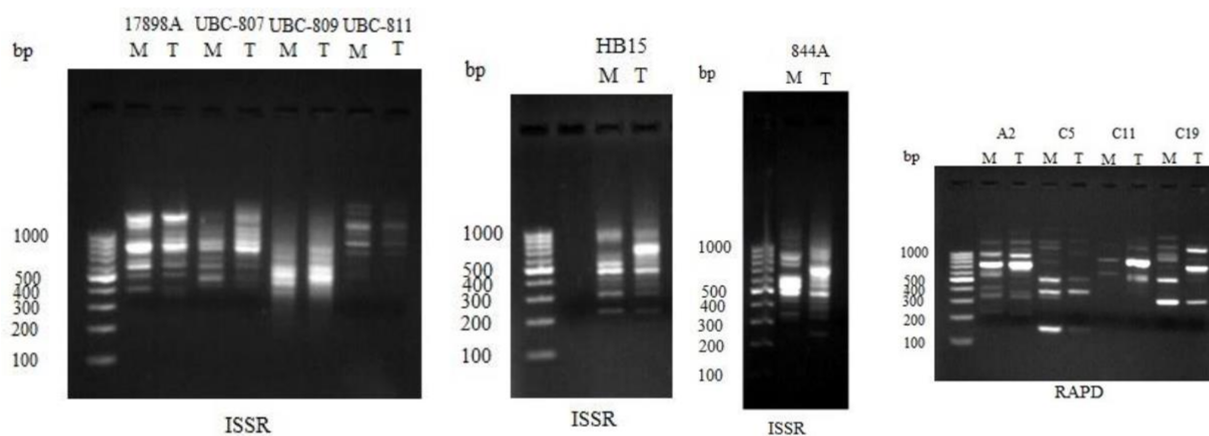

Fig. (2). ISS-PCR using six primers (17898-A, UBC-807, UBC-809, UBC-811, HB-15 and 844-A) and RAPD-PCR using four primers (A2- C5, C11 and C 19) to compare between mother and micropropagated plants of *Prunus cerassifera*., M: mother plant, T: micropropagated plant.

Random Amplified Polymorphic DNA (RAPD)

A set of ten oligonucleotides primers was pre-screened for the ability to detect polymorphism between mother and micro propagated of *Prunus cerassifera* genotype. Out of seven pre-screened primers, 4 primers successfully amplified bands and were highly informative. A number of 3 to 9 amplified DNA fragments with an average of 6 bands per primer were produced while the number of polymorphic bands ranged from 0 to 3 with an average of 1.5 polymorphic bands per primer (Table 5). A maximum number of 9 fragments were amplified with primers A-2 and the minimum number of 3 fragments were amplified with primers C-11 as shown in Table (5). The total number of reproducible fragments amplified by the 4 primers reached 24 bands, of which 7 were polymorphic fragments. This represented a level of polymorphism of 29.1%, which indicates a very high level of polymorphism among the studied genotype and treated itself.

The size of the amplified fragments also differed with the different primers and ranged from 100 to 1500 bp as shown in **Fig. (2)**. In RAPD analysis among the 4 RAPD primers used only one RAPD primer (A-02) failed to generate unique markers while the rest, 3 primers are able to generate unique markers even unique positive and(or) negative markers that could be used to identify the studied genotype. One RAPD primer produced unique negative markers (C-11). While, two primers produced, unique positive markers (C-05 and C-19) with molecular weight ranged from 300 to 1300 bp as shown in **Fig. (2)**. In the present study, RAPD analysis reveal a high level of polymorphism among the studied mother and micro propagated of *Prunus cerassifera* genotype, which enabled accurate analysis of the genetic distance. The obtained results are in good accordance with those of **Zitoun *et al.*, (2008)** who demonstrated that used primers produced reliable and reproducible banding pattern and the number, size of amplified DNA fragments and the percentage of generated polymorphic bands differed with the different primers tested. These results agree with those of **Lenka *et al.* (2015)** who demonstrated that primers produced reliable and reproducible banding pattern and that the number, size of amplified DNA fragments and the percentage of generated polymorphic bands varied among primers. Random amplified polymorphic DNA (RAPD) is a classical genetic marker technique

resulting from PCR amplification of genomic DNA sequences recognized by short random primers, firstly used by **Williams *et al.* (1990).**

Table (5): Total number of bands, number of monomorphic bands and monomorphism percentage for each used primer.

	Primer	Total number of bands	Number of polymorphic bands	Percentage of polymorphism
ISSR	17898 A	8	1	12.5
	UBC 807	8	0	0.0
	UBC 809	5	0	0.0
	UBC 811	6	1	16.7
	HB 15	7	1	14.3
	844 A	11	3	27.3
RAPD	A2	9	3	33.3
	C5	7	3	42.86
	C11	3	0	0.0
	C19	5	1	20

Conclusion

According to obtained results the best propagation protocol was established for *Prunus cerasifera in vitro* was as follows, using Clorox 15% for 10 min to get contamination-free explants, MS medium enriched with 1.5 mgl^{-1} BAP for maximum multiplication rate and MS medium with 0.4 mgl^{-1} IBA for rooting. The produced plantlets were *ex vitro* acclimatized using peat moss + perlite (1:1 v/v). So I recommend using this protocol to produce a uniform source for this important plant.

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الملخص العربي

بروتوكول فعال لإلكثار المعملي للبرقوق أرجواني األوراق (***Prunus cerasifera***)

فوزية غريب زنه

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تهدف الدراسة الحالية إلى إنشاء بروتوكول فعال لإلكثار المعملي للبرقوق أرجواني األوراق (*Prunus cerasifera*). كلوروكس بتركيز 15٪)حجم/حجم(أو كلوريد الزئبقيق بتركيز 0.1٪)وزن/حجم(لمدة 10 دقائق أعطى أفضل النتائج لكل من نسبة التعقيم)100 و90.2%(والبقاء)90 و92%(، على التوالي. تمت دراسة تأثير ثالثة أنواع من السيتوكينين)بنزايل أمينو بيورين، كى نيتين و2- أيزوبنتايل أدينين(بأربعة تراكيز)0.5، 1.0، 1.5 و 2.0(ملجم / لتر خالل مرحلة التضاعف مقارنة بالبيئة الخالية من الهرمونات. استخدام بنزايل أمينو بيورين بتركيز 1.5 مجم / لتر دفع البراعم المتكونة لتتطور إلى فريعات)نسبة تفريع 100٪(خالل ثالث نقالت متتالية. إضافة بنزايل أمينو بيورين بتركيز 1.5 أو 2.0 ملجم / لتر أعطى أعلى معدل لتضاعف الفريعات)2.13 و2.15 فريع / منفصل نباتي(على التوالي. بينما أطول الفريعات)1.92 سم(سجلت على بيئة موراشيج وسكوج مضافا اليها 1.5 مجم / لتر من 2-أيزوبنتايل أدينين. أدى إضافة 0.4 ملجم / لتر من اندول بيوترك أسيد إلى بيئة الزراعة أدت إلى تجذير 100٪ من الفريعات. استخدام البيت موس + البيراليت)1:1 حجم/حجم(كوسط نمو ألقلمة النباتات خارج المعمل أدى الى أفضل النتائج لنسبة البقاء، االرتفاع، الوزن الطازج والجاف لسوق النبياتات المؤقلمة. استخدمت قطع الحمض النووي المكبرة بادئات A-844)لـ ISSR(وA2)لـ RAPD(للمقارنة بين النبات األم والمكثرة معمليا. أظهرت البيانات أن الحد األقصى لعدد الحزم)11 و 9(على التوالي لبادئات الحمض النووي ذات حجم جزيئي يتراوح بين 226.98 - 1007.92 و 215.16 - 1619.62 زوج قاعدي (bp). أشارت البيانات إلى أن النطاق الوراثي ضيق جدا بين النباتات المكثرة معمليا والنبات األم وأن بروتوكول التكاثر الدقيق آمن وراثيًا.