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STUDIES ON PROPAGATION OF *ACIDANTHERA MURIELAE* PLANTS BY USING TISSUE CULTURE TECHNIQUE

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ABSTRACT

This study was intended to find out a well-defined commercial production protocol for *in vitro* propagation of *Acidanthera murielae*. In this respect, buds from *Acidanthera* corms were effectively surface sterilized using sodium hypochlorite solution at 2% for 20 min. MS medium supplemented with 2 mg/l IBA (Indole Butyric Acid) was the best medium for shoot length and number of leaves promotion during the establishment stage. For further multiplication, MS medium supplemented with 1 mg/l BA and 2 mg/l Kin formed the highest number of shoots. For *in vitro* rooting, MS medium at half strength supplemented with 2.0 mg/l IBA was suitable for root formation. The plantlets were transferred to a mixture of peatmoss:sand at a ratio of 1:1 with great success for *ex vitro* acclimatization inside the greenhouse conditions.

Key words: *Acidanthera*, Cormel, *In vitro*,Micropropagation, Peacock Orchid, Shoot tips, Tissue culture.

INTRODUCTION

Acidanthera murielae (Peacock Orchid) is a great staple flower for garden beds or patio pots. It's also ideal as a cut flower as it lasts so long in a vase. This lovely scented plant of the Gladiolus Callianthus Genus (Family Iridaceae). *Gladiolus acidanthera* is a beautiful South African wild flower. This Gladiola blooms in mid-summer. The flowers have a strong, sweet fragrance and make wonderful bouquets. The flower color is white **(Piskornik *et al*., 1989)**.

Armitage and Laushman (1990) studied that corms of *Acidanthera murielae* were planted during the autumn and winter of 1985, in raised beds covered with a depth of 0.6-0.8 m of a clay loam/horse manure mixture, in an attempt to extend the harvest periods of cut flowers.

An efficient tissue culture protocol of Gladiolus was developed to multiply the cormels. Callus was initiated in the cultures from middle and bottom slices of the cormels. The maximum callus induction was observed on MS medium supplemented with 4 mg/l NAA while best proliferation of shoots was recorded on MS medium supplemented with 0.5 mg/l NAA. The efficient shoot regeneration from proliferated calli was observed in 4 mg/l BAP. Root formation was induced on MS medium supplemented with IBA and 3-5% sucrose **(Memon *et al*., 2014)**.

The best protocol for mass scale *in vitro* corm production of Gladiolus was liquid culture and coconut coir explored as a matrix. The culture was initiated from the basal portion of the innermost leaf of the sprout. The cut surface of responding explant was swelled in MS solid basal medium supplemented with 2 mg/l NAA. The shoots were observed when the explant was transferred in MS medium containing 2 mg/l BAP and 0.2 mg/l NAA **(Bera *et al*., 2015)**.

Cormel tip of gladiolus (*Gladiolus* x *grandifforus* L.) was used as explant for *in vitro* regeneration on MS media supplemented with different plant growth regulators. Maximum callus formation was observed on MS medium supplemented with 4.0 mg/l 2,4-D. The inducted calli were subcultured on MS medium supplemented with 4.0 mg/l BAP and 0.5 mg/l NAA for best shooting followed by 1/2 MS medium fortified with 3.0 mg/l IAA for rooting. Maximum and earliest formation of cormels was obtained on MS medium which contained 4.0 mg/l NAA plus 6% sucrose with good cormel size. Decrease in metabolities like starch, reducing sugars and total phenols while, increase in total soluble proteins was found during shoot and root differentiation. Activities of enzymes, viz. polyphenol oxidase and peroxidase increased during shoot and root differentiation. It was proved that metabolic and enzymatic activity during *in vitro* morphogenesis led to rapid organogenesis and multiplication **(Koushik Dutta and Gantait, 2016)**. Corm slice explant of gladiolus (*Gladiolus hybridus*) was cultured on MS medium with different combinations of plant growth regulators. The best medium for callus initiation was MS+2.0 mg/l BAP+0.5 mg/l 2,4-D+30.0 g/l sucrose+7.5 g/l agar. MS medium supplemented with BA at 2.0-3.0 mg/l in combination with 0.5 mg/l NAA, 30.0 g/l sucrose and 7.5 g/l agar induced higher shoot proliferation. *In vitro* rooting with higher number of

root and root length were recorded on MS medium MS+0.5 mg/l IBA+15.0 g/l sucrose+7.5 g/l agar. Normal plantlets were transferred to pots and hardened in on Environmental Growth Cabinet and transferred to field successfully **(Tripathi *et al.*, 2017)**.

Callus was induced from Gladiolus corms on MS medium and 0.5 mg/l NAA+0.5 mg/l BAP. The callus differentiated into embryos on 2,4-D, while the addition of 1.0 mg/l BAP and 0.25 mg/l NAA was fine for proliferating embryos. Direct somatic embryos were formed on corm surfaces with 0.5-1.0 mg/l 2, 4-D. The amendment of GA_3 was more responsive compared to ABA and GA_3 added together. The mature embryos were converted into plantlets on MS medium supplemented with 0.5 mg/l BAP **(Mujib *et al.*, 2017)**. Cormel tips and corm apical bud explants gave best shoot regeneration on MS medium supplemented with 4.0 mg/l BAP and 4.0 mg/l kinetin. The best rooting was observed on 1/2 MS medium supplemented with 4.0 mg/l NAA **(Koushik Dutta *et al.*, 2010).** The shoots in *Gladiolus sp*. produced roots on MS with 1.0 mg/l IBA and 6% sucrose. After five weeks, rooted plantlets were transferred for initiation of cormels. The protocol enabled to harvest more than 50,000 cormels within 150 days starting from a single *in vitro* root explant **(Arvind and Kumar, 2012)**. MS medium supplemented with 2 mg/l Kinetin had efficiency for regeneration of gladiolus cormlets. Cormlets cultured on MS medium containing 2 mg/l GA_3 showed the best proliferation. MS medium supplemented with 2 mg/l NAA+2 mg/l IBA initiated rooting **(Maitra *et al.*, 2011)**. Multiplied corms were successfully acclimatized on a medium containing soil:sand:brick pieces at 1:1:1 ratio. Acclimatized cormlets were established on two different potting mixtures, i.e. sand:leaf mould:brick pieces (1:1:1) or sand:compost:brick pieces (1:1:1). The highest sprouting percentage was observed on a potting mixture containing sand, leaf mould and brick pieces (1:1:1) under greenhouse conditions **(Dharmasena *et al.*, 2011)**.

Presence of GA_3 along with auxin and cytokinin promoted the elongation of shoots. The plantlets had the maximum length of shoot in the medium containing 8.88 micro M BAP+9.29 micro M Kin+1.07 micro M NAA+0.58 micro M GA_3. The regenerated shoots were separated and transferred to the medium containing 1/2 MS+4.92 micro M IBA for rooting **(Pragya Singh, *et al.*, 2012)**.

The aim of this study was to establish a protocol for *in vitro* propagation of *Acidanthera muriela* plant for commercial production. So, the experimental trial was carried out mainly by using different concentrations of sodium hypochlorite (NaOCl) solution and different salt

strengths of MS-medium, and also by manipulating growth with the use of various concentrations of Kin, BA and IBA. Different concentrations of peatmoss and sand were investigated for the adapting behavior later on during acclimatization.

MATERIALS AND METHODS

1. Location and duration:

This study was carried out in the laboratory of Tissue Culture, Zohria Botanical Garden, Cairo, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture. The experiments were carried out through 2018 – 2019 years.

2. Plant material:

The mother plants of *Acidanthera murielae* were imported from Holland. The parts used as explants were buds from corms.

3. Culture medium:

Murashige and Skoog (MS) basal medium was used for culturing the explants. The medium was supplemented with 30 g/l sucrose and 8 g/l agar. It was adjusted to pH 5.7±0.1 and autoclaved at 121°C (1.5 kg/cm^2) for 20 min before being used.

4. Experimental treatments:

Surface sterilization of buds:

The aim of this experiment was to study the effect of sodium hypochlorite (NaOCl) solution at 1.0, 2.0 and 3.0 % on surface sterilization of *Acidanthera* buds *in vitro*.

Buds of *Acidanthera* were excised from the corms, initially 0.5-1.0 cm in length then were washed by soapy water for 10 minutes followed by 45 minutes under running tap water. The buds were then sterilized by immersion in NaOCl (commercial ‹Clorox' solution) at 1.0, 2.0 and 3.0 % containing 2-3 drops of Tween-20 for 15 and 20 minutes. Finally, explants were washed 5 times with sterile distilled water. Each sterilized explant was cultured separately under sterile conditions in a 350 ml jar.

For surface sterilization of explants, six treatments were carried out, each treatment consisted of 10 jars and each bud was cultured separately in a jar. The following data were studied: survival percentage, contamination percentage and mortality percentage.

Effect of IBA on buds establishment:

Murashige and Skoog (MS) medium was used as culture medium supplemented with Indole butyric acid (IBA) at 0.0, 1.0, 2.0 and 3.0 mg/l. For establishment stage, four treatments were studied and each treatment consisted of 3 jars. The following data were studied: shoot length (cm) and number of leaves.

Effect of BA and Kin on multiplication stage:

For the multiplication stage, 20 treatments were studied using BA at 0.0, 0.5, 1.0, 1.5 or 2.0 mg/l and Kin at 0.0, 1.0, 2.0 and 3.0 mg/l for three subcultures. The following data were studied: number of shoots/ shoot, shoot length (cm) and number of leaves/shoot.

Effect of IBA and MS-medium strength on rooting growth:

In the rooting stage, 12 treatments including combinations of 4 IBA levels (0.0, 1.0, 2.0 or 3.0 mg/l) and three strengths of MS-medium (full, ½ MS or ¼ MS) were used. The following data were studied: shoot length (cm), number of leaves, number of roots and root length (cm).

Acclimatization stage:

Rooted plantlets were pricked out singly into 8 cm plastic pots filled beforehand with 1: 0, 1: 1, 1: 2 and 1: 3 (v/v) peatmoss and sand, respectively. Pots were covered with clear transparent plastic sheets to maintain high humidity of cultures for two weeks, then they were gradually removed to reduce humidity and to adapt plantlets to greenhouse conditions. The following data were studied: plantlet height (cm) and number of leaves.

Experimental design and statistical analysis:

A complete randomized design was employed in all of the experiments. Analysis of variance was used to show the least statistical differences between treatments using the L.S.D at 5% probability level **(Snedecor and Cochran, 1989)**.

RESULTS AND DISCUSSION

1. Effect of sodium hypochlorite concentrations on surface sterilization of *Acidanthera* buds *in vitro*:

Concerning the effect of sodium hypochlorite (NaOCl) solution at 1.0, 2.0 and 3.0 % on survival percentage, regardless of the effect of different time periods of treatment. Result data shown in **Table (1)** indicate that the percentage of survival of explants was increased by

increasing the NaOCl to 2% concentration. The highest survival percentage was recorded with 2 % NaOCl followed by that recorded with 3 % NaOCl (45 and 30 % survival, respectively). Meanwhile, the highest percentage of contamination (50%) was observed using 1 and 2% NaOCl. The treatment which decreased the mortality to 0 and 5% was also at 1 and 2% NaOCl. On the other hand, the significantly lowest survival percentage (10%) was recorded with 1 % NaOCl.

Concerning the effect of soaking period treatments, the data indicated that increasing the soaking period of explants increased the survival, and thus affected contamination and mortality percentage of explants. The highest percentage of survival (33%) and the lowest percentage of survived explants (23%) was recorded when the explants were immersed for 20 and 15 min, respectively.

Using 2 % NaOCl and the explant immersed for 20 min resulted in the highest survival percentage of explants (70 %) and showed at the same time a low percentage of contamination and mortality (30 and 0%, respectively). These results are in agreement with those obtained by **(El-shamy, 2009)** when NaOCl and soaking periods were used on *Amphilophium paniculatum*.

Table (1): Effect of different concentrations of NaOCl and soaking periods on surface sterilization of *Acidanthera murielae* buds.

NaOCl (%)	Survival (%)			Contamination (%)			Mortality (%)		
	Min		Mean	Min		Mean	Min		Mean
	15	20	(A)	15	20	(A)	15	20	(A)
1	10	10	10	90	90	90	00	00	00
2	20	70	45	70	30	50	10	00	5
3	40	20	30	40	30	35	20	50	35
Mean(B)	23	33		67	50		10	17	
$LSD_{0.05}$									
Clorox (A)	6.88			5.62			3.44		
Periods (B)	5.96			4.87			2.98		
(AxB)	11.92			9.75			5.96		

2.Effect of IBA on establishment of *Acidanthera* buds:

For the establishment stage, results in **Table (2)** and Plate (1) indicated that the best medium treatment was MS supplemented with 2 mg/l IBA. After four weeks, 2 mg/l IBA were observed to be better than other concentrations of IBA in both number of leaves and shoot length (5.33cm and 1.17 leaves, respectively). Notably, 2 and 3mg/l IBA exhibited no significant differences between them (5.33 and 5.67 cm, respectively) when comparing their LSD values in shoot length.

These results are in disagreement with that obtained by **Choudhary, *et al.*, 2010** who found that cormel slices as explants of *Gladiolus grandiflora* was placed on a shoot induction medium containing MS medium and only 0.5 mg/1 BAP for decent shoot induction.

Table (2): Effect of different concentrations of IBA on establishment of *Acidanthera murielae* buds.

IBA	Shoot length (cm)	No. of leaves
0.0	2.33	0.50
1.0	3.33	0.67
2.0	5.33	1.17
3.0	5.67	0.83
$LSD_{0.05}$	0.54	0.24

3.Effect of BA and Kin on multiplication of *Acidanthera* shoots:

All individual shoots, which have resulted initial establishment medium treatments were cultured again for the multiplication stage. Uniform individual shoots one cm in length with one leaf were cultured on the multiplication medium and kept for three subcultures.

Number of shoots/shoot:

For BA concentrations, data presented in **Table (3)** and Plate (1) show that BA concentrations induced number of shoots/shoot. Results showed that the shoots were increased and the highest number of shoots/shoot (22.5 shoots) was obtained at 1.0 mg/l BA. There were significant differences in number of shoots/shoot between the different concentrations of BA and control treatment.

Similarly, as in BA, number of shoots/shoot was significant and led to additive increases in number of shoot/shoot. The largest number of shoots/shoot was found (21.4 shoots) at 2.0 mg/l Kin and this concentration was significant when compared with the other remaining treatments. The lowest number of shoots/shoot was found (7.1 shoots) in the control treatment.

Results of the interaction indicated that number of shoots/shoot was increased due to both BA and Kin concentrations together. The highest number of shoots/shoot was found (34.7 shoots) at 1.0 mg/l BA and 2.0 mg/l Kin. There were significant differences between almost all the different treatments when compared with the control treatment.

The results are in harmony with that obtained on gladiolus. Where, MS medium supplemented with BA at 2.0-3.0 mg/l in combination with

0.5 mg/l NAA, 30.0 g/l sucrose and 7.5 g/l agar induced higher shoot proliferation with **Tripathi *et al*., (2017).**

Shoot length (cm):

Data calculated in **Table (3)** reveal that supplementation of MS medium with BA at 0.0, 0.5, 1.0, 1.5 and 2.0 mg/l decreased significantly shoot length. While the highest value of shoot length (7.5 cm) was formed at the control treatment. Whereas, the shortest shoot was 4.5 cm at 2 mg/l BA.

Concerning the effect of Kin, Kin produced higher shoot length than the control treatment. The longest shoot (6.2 cm) was recorded on a medium supplemented with 3 mg/l Kin when compared with the control treatment (4.9 cm).

Regarding the interaction between BA and Kin, when the shoots were cultured on a medium supplemented with 2 or 3 mg/l Kin they produced the highest shoot length (8.3 and 8.7 cm, respectively) with no significant differences between them when compared with most of the remaining treatments.

Number of leaves/shoot:

Results presented in **Table (3)** indicate that BA at different concentrations decreased number of leaves/shoot. The control treatment was significantly higher when compared with the other BA treatments. The highest number of leaves/shoot was 3.3 leaves by the control treatment. While, the lowest number of leaves/shoot was found to be 1.2 leaves at 2 mg/l BA.

For Kin concentrations, results showed that Kin increased number of leaves/shoot. Thus, it was found that the highest number of leaves/shoot was obtained at 3 mg/l Kin (2.3 leaves) when compared to the other treatments.

Concerning the interaction between BA and Kin, it was evident that Kin with zero-level BA increased significantly number of leaves/shoot. Thus, the greatest number of leaves/shoot (4.3 leaves) was obtained at 3 mg/l Kin without BA when compared to the other treatments.

Table (3): Effect of different concentrations of BA and Kin on some growth parameters during the multiplication stage of *Acidanthera murielae*.

BA (mg/l)	Number of shoots/shoot					Shoot length (cm)					Number of leaves/shoot				
	Kin (mg/l)					Kin (mg/l)					Kin (mg/l)				
	0.0	1.0	2.0	3.0	Mean (A)	0.0	1.0	2.0	3.0	Mean (A)	0.0	1.0	2.0	3.0	Mean (A)
0.0	1.3	4.7	16.7	12.3	8.8	6.3	6.7	8.3	8.7	7.5	2.3	2.7	3.7	4.3	3.3
0.5	7.3	11.7	22.7	16.3	14.5	4.7	5.7	6.3	6.3	5.8	1.3	1.5	1.7	2.3	1.7
1.0	9.7	26.0	34.7	19.7	22.5	5.0	5.3	5.7	5.7	5.4	1.3	1.3	1.7	2.0	1.6
1.5	8.7	11.7	18.3	12.3	12.8	4.3	4.3	4.7	5.3	4.7	1.0	1.3	1.3	1.7	1.3
2.0	8.3	10.3	14.7	9.3	10.7	4.0	4.3	4.7	5.0	4.5	1.0	1.0	1.3	1.3	1.2
Mean (B)	7.1	12.9	21.4	14.0		4.9	5.3	5.9	6.2		1.4	1.7	1.9	2.3	
$LSD_{5\%}$															
BA (A)			0.98					0.46					0.46		
Kin (B)			0.93					0.41					0.23		
AxB			1.96					1.91					0.61		

4.Effect of IBA and MS-medium strength on rooting of *Acidanthera* shoots:

All individual shoots, which have resulted from the multiplication stage were cultured for the rooting stage. Uniform individual shoots 1.5 cm in length with two leaves were cultured and kept for four weeks.

Shoot length (cm):

Concerning the effect of IBA concentrations, results in **Table (4)** indicated that the addition of IBA to the rooting medium led to increases in shoot length. It was found that 2 mg/l IBA gave the highest shoot length (4.5 cm) and there were no significant differences between it and 3 mg/l IBA (4.8 cm).

For MS-medium strength, results were found that the highest shoot length (4.8 cm) was recorded at half-strength MS medium. While, the shoot length was decreased at full and quarter strengths (3 and 3.5 cm, respectively) and there were no significant difference between them.

For the interaction between IBA and MS-medium strength, by increasing the IBA concentration to 2 mg/l at half-strength MS medium, there was a marked increase in shoot length (6 cm) and there were no significant difference between 2 and 3 mg/l IBA (6 and 6.5 cm, respectively) at half-strength MS medium.

Number of leaves:

It is clear from the data in **Table (4)** that the various concentrations of IBA affected significantly number of leaves/shoot. The highest number

of leaves (3.5) was obtained on medium supplemented with 2 mg/l IBA. The lowest number of leaves (2.3) was obtained on the control treatment.

MS medium at half strength was increased significantly number of leaves (3.5) in greatest number of leaves. There were significant differences between MS at half strength and the different strength medium when compared with LSD.

The interaction between IBA and MS-medium strength demonstrated that half MS medium supplemented with 2 mg/l IBA produced the highest number of leaves (4.5) and there were significant difference between it and most treatments.

Table (4): Effect of different concentrations of IBA and MS-medium strength on shoot length (cm) and number of leaves during *in vitro* rooting of *Acidanthera murielae*.

MS-strength / IBA(mg/l)	Shoot length (cm)				Number of leaves			
	MS	1/2MS	1/4MS	Mean (A)	MS	1/2MS	1/4MS	Mean (A)
0.0	2.0	2.5	2.0	2.2	2.0	3.0	2.0	2.3
1.0	3.0	4.0	3.5	3.5	2.5	3.0	3.0	2.8
2.0	3.5	6.0	4.0	4.5	3.0	4.5	3.0	3.5
3.0	3.5	6.5	4.5	4.8	3.0	3.5	3.5	3.3
Mean (B)	3.0	4.8	3.5		2.6	3.5	2.9	
LSD at 5% IBA (A)			0.93				0.76	
MS-medium strength (B)			0.70				0.52	
AxB			1,46				0.98	

Number of roots:

Results recorded in **Table (5)** showed that number of roots/shoot was increased due to raise of IBA concentrations, giving the highest number of roots (10) at 3 mg/l IBA. While, the lowest number of roots (2.9) was obtained by the control treatment.

A similar trend was obtained as a result of MS-medium strength giving the highest number of roots (11) on the medium at half strength which was higher than full and quarter strength (3.7 and 6.6 roots, respectively).

As for the interaction between IBA and MS-medium

strength, it was found that the greatest number of roots (15) was obtained at 3 mg/l IBA and half strength medium. While, the lowest number of roots (1.2) was obtained at control treatment with full medium strength.

Root length (cm):

Result data in **Table (5)** and Plate (1) showed that IBA clearly affected the root length. Medium supplemented with 2 mg/l IBA gave the longest root (6.7 cm). The shortest root (1.7 cm) was obtained by the control treatment.

For MS-medium strength treatments, the results indicated that half MS-strength gave the longest root (6.1 cm) whereas, full MS-strength gave the shortest root (2.8 cm) and there were significant differences between all medium strength.

With regards to the interaction between IBA concentrations and MS-medium strength on root length, it was found that half strength of MS-medium supplemented with 2 mg/l IBA gave the best positive response (9.5 cm) for root length when compared with the other remaining treatments.

These results are more less in harmony with those results obtained on gladiolus, which seems to favor half strength MS medium for the rooting stage **(Akhare, *et al*. 2007)**.

Table (5): Effect of different concentrations of IBA and MS-medium strength on number of roots and root length (cm) during *in vitro* rooting of *Acidanthera murielae*.

MS-strength / IBA(mg/l)	Number of roots				Root length (cm)			
	MS	1/2MS	1/4MS	Mean (A)	MS	1/2MS	1/4MS	Mean (A)
0.0	1.2	5.3	2.4	2.9	1.0	2.5	1.5	1.7
1..0	3.6	8.9	6.5	6.3	2.5	3.5	3.0	3.0
2.0	5.8	14.7	8.6	9.7	3.5	9.5	7.0	6.7
3.0	6.2	15.0	8.9	10.0	4.0	9.0	6.0	6.3
Mean (B)	3.7	11.0	6.6		2.8	6.1	4.4	
LSD at 5% IBA (A)			0.11				0.26	
MS-medium strength			0.09				0.20	
AxB			0.19				0.45	

5. Acclimatization stage:

During this stage of culture as a pointed out in **Table (6)** and Plate (1) plantlets grew steadily but slowly and had healthy appearance. Plantlets cultured in a mixture of peatmoss:sand (1:1) gave highest plantlet length and number of leaves (20 cm and 10 leaves, respectively) when compared with other cultured mixes used. After four weeks, no abnormalities in physical appearance and growth habits were observed on the transplanted plants. All plants survived when plantlets were cultured under greenhouse conditions after the acclimatization stage.

Here, results of acclimatization agree with what has been reported with gladiolus where plantlets were transplanted with highest survival percentage (100%) in soilrite medium and then keeping the pots in a mist chamber (**Akhare, *et al*. 2007)**.

Table (6): Effect of different mixtures of peatmoss and sand on *ex vitro* acclimatization stage of *Acidanthera murielae.*

Peatmoss	Sand	Plantlet height (cm)	Number of leaves
1	0	12	5
1	1	20	10
1	2	15	7
1	3	14	6
$LSD_{0.05}$		4.8	3.2

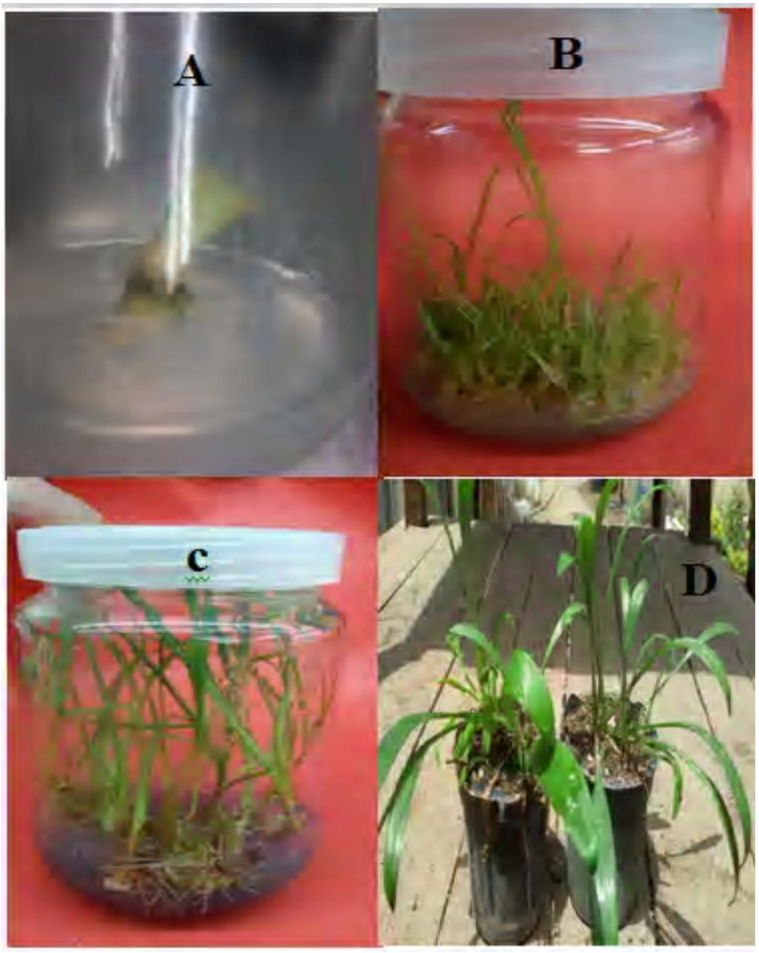

Fig . (1): *In vitro* micropropagation of *Acidanthera murielae*
A. Establishment stage.
B. Multiplication stage.
C. Rooting stage.
D. Acclimatization stage.

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دراسات على إكثار نباتات *Acidanthera Murielae* بواسطة تكنيك زراعة الأنسجة

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أجريت هذه الدراسة خلال الفترة من سنة 2018 - 2019 فى معمل زراعة الأنسجة بحديقة الزهرية التابعة لمعهد بحوث البساتين-مركز البحوث الزراعية-وزارة الزراعة-جمهورية مصر العربية.

كان الهدف من هذه الدراسة هو تحديد البروتوكول التجارى لإكثار النبات والمعروف بالاسم الدارج (أوركيد الطاووس) عن طريق زراعة الأنسجة. ويمكن تلخيص أهم النتائج التى تم التوصل إليها فى الأتى:

أمكن إكثار النبات بواسطة البراعم الطرفية للأبصال كمنفصلات نباتية حيث تم تعقيمها بواسطة هيبوكلوريت الصوديوم بتركيز 2 % لمدة 20 دقيقة كأفضل تركيز للتعقيم السطحى للمنفصلات. وفى مرحلة التأسيس أستخدمت بيئة موراشيجى وسكوج بالإضافة إلى إندول حمض البيوتريك بتركيز 2 مجم / لتر كأفضل بيئة من حيث طول الأفرع وعدد الأوراق. أما فى مرحلة التضاعف فقد أستخدمت بيئة موراشيجى وسكوج المضاف إليها البنزيل أدينين بتركيز 1 مجم/ لتر والكينتين بتركيز 2.0 مجم / لتر كأفضل بيئة من حيث عدد الأفرع. وفى مرحلة التجذير أستخدمت بيئة موراشيجى وسكوج بتركيز نصف قوة للأملاح والمضاف إليها إندول حمض البيوتريك بتركيز 2.0 مجم / لتركأفضل بيئة من حيث عدد وطول الجذور. وفى مرحلة الأقلمة أستخدم خليط من البيتموس والرمل بنسبة 1:1 لإعطاء أعلى نسبة نجاح داخل الصوب.