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GREEN GLOBULER BODIES INDUCATION OF *Platycerium bifurcatum* THROUGH TISSUE CULTURE

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ABSTRACT

Plantlets of Platycerium bifurcatum were successfully regenerated from leaf section via callus formation, GGB (green globular bodies) production as intermediate stage and GGB segments were proliferation into shoots.

Fraible callus induction in leaf section was achieved on full strength MS medium supplemented with 1 mg/L^{-1} 2,4- D+1mg/l kinetin within two subculture (every 4 weeks). The maximum production of GGB was obtained by the addition of 3 mg/l BA to MS medium. Segments of GGB were cultured on MS medium containing relatively low concentration of NAA (0.0 to 0.9 mg/l) and regenerate to larger shoot numbers than GGB segments which were cultured on medium containing relatively high concentrations of auxins NAA, IBA, IAA (5,10 and 15 mg/l).

The greatly shoot numbers production from GGB segments when 0.9 mg/L^{1-} NAA was added to the medium. Higher growth value and shoot numbers were observed on GGB segments by using mediuum supplemented with 0.9 mg/l NAA, solidified by 3.0 mg/l agar and adjusted pH to 5.2. Also, the effect of agar concentration and pH value were examined. Shoots were planted in plastic pots containing peatmoss sand mixture (3:1v/v) during acclimatization in greenhouse.

INTRODUCTION

Platycerium bifurcatum is an important fern used for the indoor beautification. It is usually propagated by spores or offsets, The propagation by spores and offsets is relatively slow because: (1) The plant develops few offsets and (2) developing marketable plants from spores requires about two years (Nagy, 1986). In contrast, *in vitro* propagation of plants enabled to obtain numerous plantlets within a short period of time (Cooke, 1979; Padhya and Mehta, 1982 and Higuchi *et al*., 1987). Most studies of callus induction and its differentiation to plantlets were performed on seed plants (Thrope, 1988), but very little were made on fern (Mahabale and Patankar, 1980; Caponetti *et al*., 1982 and Byrne and Caponetti, 1992).

The importance of callus differentiation to green-globular bodies (GGB) was reported, in *Platycerium,* by Thentz and Moncousin (1984); Camloh and Gogala (1991); Camloh *et al*. (1994); and Kwa *et al*. (1997a).

The influence of different auxins concentration on growth and development of ferns was extensively studied by Garcia *et al*., 1987 on *Cyrtomium*; Salome *et al*., 1987 on *Adiantum*; Wei, 1998 on *Nephrolepis*, but little was made on *Platycerium* (Kwa *et al*., 1997b).

Among factors affecting the micropropagation of ferns is MS (Murashige and Skoog,1962)-salt strength as well as agar concentration and pH value of the culture media. The influence of MS-salt strength was investigated by Camloh *et al*. (1989); Amaki and Higuchi (1990). Also, the

effect of agar level and pH value on the propagation of *Platycerium in vitro* was reported by Thentz and Moncousin, 1984; Camloha and Gogala, 1992 and Pevalek-Kozlina, 1996.

This work aimed to investigate the effect of MS-salt strength, auxin and cytokinin levels, agar concentration, and pH value on the propagation of *Platycerium bifurcatum in vitro.* The producing plantlets were hardened-off and acclimatized in peatmoss sand mixture (3:1v/v), under greenhouse conditions, to obtain healthy vigorous plants.

MATERIALS AND METHODS

This work has been carried out in Tissue Culture Laboratory, Agricultural Development Systems (ADS) Project (Giza, Egypt), during the period 1995 to 1998. Stock imported plants of *Platycerium bifurcatum,* obtained from Netherland, served as the source material for leaf sections. Fully mature leaves were washed by soap and water before submerging in a solution of 2% sodium hypochorite plus 0.1% tween 20 for 20 min. The leaves were then rinsed three times in sterilized distilled water. The damaged base, outside edges and tips of leaves were removed. The remainder leaf sections were cut into 0.5 × 0.5cm, under aseptic conditions, and used as initial explants.

To investigate the influence of 2,4- dichlorophenoxy acetic acid (2,4-D) and kinetin on callus formation by *Platycerium* leaves, explants were horizontally embeded on MS-basal media (Murashige and Shoog, 1962) supplemented with 2,4-D and kinetin at levels of 0.0, 0.5, 1.0 and 2.0 mg/L^{-1} respectively. Thereafter, callus formation (%) was calculated after one (4 weeks) and two (8 weeks) subcultures.

The effect of MS salt strength on survival (%) and callus formation (%) was studied by using media supplemented with1 mg/ L^{-1} 2,4-D/l plus 1mg kinetin/l and containing MS-basal salts at the levels of MS, 1/2 MS, and 1/4 MS and 1/8 MS. Then, both parameters were recorded after one and two subcultures.

For callus differentiation into green-globular bodies (GGB), explants were cultured on MS-basal media contained 0.0, 3.0 and 5.0 mg BA/mg L^{-1}. After 8 and 16 weeks post culturing, GGB (%) formation was recorded.

Leaf sections (0.5cm) formed green globuler bodies (GGB) produced from the previous medium (MS + 3 mg/l BA) were used for studing the influence of auxin level on shoot multiplication rate of *platycerium,* Indole acetic acid (IAA), Indole buytic acid (IBA) or Naphthalene acetic acid (NAA) were added to MS- basal medium at rates of 0.0, 5.0, 10.0 and 15.0 mg/ L^{-1}. Then, some culture criteria (table, 4_A) were recorded after 4 weeks. In a terminal experiment the effect of NAA level (0.0, 0.1, 0.3, 0.5, 0.7, and 0.9mg /L^{-1}) on shoots number per culture and leaves number per culture was studied after 4 weeks.

Liquid media with pH values of 4.2, 4.7, 5.2, 5.7 and 6.2 were solidified by adding Difco-Bacto agar (0.0, 3.0, 5.0, 7.0 and 9.0gL^{-1}), then GGB explants were cultured for 8 weeks on the best previous media supplemented with 0.9 mgL^{-1} NAA. Shoots number and leaves per culture,

and growth value per culture were recorded. *Growth value was estimated and presented as described by (Ziv, 1992).

In all cases, media (pH 5.7) were dispensed into 2.5 × 15cm culture tubes (20ml/tube), autoclaved for 20 min at 121°C and 1.2 kg cm^{-2}, then cooled and kept for 4-15 days before use. All cultures were incubated at 27±2°C and 2 K-lux ($16h^{-1}$ day) provided by cool white-fluorescent lamps.

During acclimatization stage, shoots were transplanted in plastic pots containing peatmoss sand mixture (3:1v/v) and were hardened-off under greenhouse conditions to obtain healthy vigorous plants used for the indoor beautification.

All experiments were repeated twice, under controlled conditions, and conducted by using a completely randomized design in factorial arrangement with 9 replicates. All data were averaged and statistically analyzed by using one-and two-way analysis of variance. In case of percentages, the original data were firstly arcsine-transformed prior to statistical analysis. The least significant difference (L.S.D. at 5%) was used to compare between means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Data presented in table (1) clearly show the effect of different levels of 2,4-D and Kinetin on callus formation of *Platycerium bifurcatum* leaf sections after one and two subcultures

MS basal medium supplemented with 2,4-D and Kinetin served as effective media for callus production in *Platycerium.* The greatest values were obtained with the 2,4-D: Kinetin at (0.5: 0.5 and 1:1 mgL^{-1}), the average were 70.3 and 83.3%, respectively after two subcultures.

Callus morphology also differed, dense green pigmented callus was produced on (0.5:1.0, 0.5:2.0, 1.0:2.0 mg L^{-1}) 2,4-D: Kinetin media, friable callus produced on 2,4-D : Kinetin at 1:1 mg L^{-1} medium. Browning, most likely due to oxidized polyphenols appeared on high concentration of kinetin after one month. Browning was not associated with any particular concentration or ratio of 2,4-D: Kinetin. Avery small amount of callus tissue was produced on MS basal medium supplemented with kinetin or 2,4-D alone (Table,1). Kwa *et al* (1997a) initiated callus successfully from gametophytes of *Platycerium coronarium* when cultured on MS medium in the prescence of 2,4-D. This callus was soft and green. In the absence of 2,4-D, no callus developed. The authors found that, the callus which arose from cell cultured on medium with kinetin, proliferated when cultured on MS medium with 2µM 2,4-D. Kwa *et al* (1997b) reported that, the callus initiated from the gametophytes of *Platycerium coronarium* cultured on MS medium with 2% sucrose and 20 µM 2,4-D was soft and green.

*** Growth value (GV) is expressed as a value calculated by dividing the differnce between final and initial fresh weight (FW) by the initial FW: (FW final- FW initial / FW initial).**

Table (1) : Effect different levels of 2,4-dichlorophenoxy acetic acid (2.4-D) and Kinetin on callus formation (%) by *Platycerium bifurcatum* leaf sections after one and two subcultures. Leaf sections were cultured *in vitro,* for 4 weeks, on full-strength MS-basal medium.

Kinetin Level mg/L^{-1}	After one subculture				After two subcultures			
	2.4-D (mg/L^{-1})				2.4-D (mg/ L^{-1})			
	0.0	0.5	1.0	2.0	0.0	0.5	1.0	2.0
0.0	0.00i	8.30gh	12.5 0fg	16.60ef	0.00k	20.80i	29.10h	20.80i
0.5	0.00i	50.00b	33.75c	37.50c	4.20jk	70.30b	54.20de	50.00ef
1.0	0.00i	25.00d	66.60a	20.80de	4.20jk	41.60g	83.30a	58.30cd
2.0	4.20 hi	20.80de	33.30c	37.50c	8.30j	45.80fg	58.30cd	62.50c

Means followed by different letters are significantly different at the 5% level according to L.S.D.

Data presented in Table (2) indicated that, the increase in concentration of MS salt strength produced an increase in survival and callus formation after one and two subcultures. The main factor influencing callus formation was the salt concentration of the medium. These results are in line with Higuchi and Amaki (1989) who found that, on the full strength MS medium, the GGB attained to an increase the weight after 14 weeks. Dilution of MS medium reduced the growth of GGB with 25% of the original prescription. The authors added that the growth of GGB of *Asplenium nidus* was reduced to the about the half.

The best concentrations were 3 and 5 mg/L^{-1} BA for increasing the green- globular bodies (GGB) formation, respectively (Table 3).Data revealed that BA at the concentration of 3mg/L^{-1} showed the high callus differentiation to green globular bodies (GGB). It was 58.3 and 83.3 after 8 and 16 weeks, respectively. These results are in line with Thentz and Moncousin (1984) who reported that bud induction on *Platycerium bifurcatum* leaves was successful when BAP was added to the medium. Also, rapid propagation of *Asplenium nidus* L was achieved by means of rhizome segment culture *in vitro*. Rhizome segments produced green globular bodies (GGB) on Murashige and Skoog medium supplemented with 2.2 µM of benzylaminopurine. GGB were rapidly multiplied on the BAP medium as indicated by Higuchi and Amaki, (1989). Camloha *et al* (1994) showed that, BA in the medium resulted in a higher frequency of multicellular scale development and bud organogenesis, however, the buds of *Platycerium bifurcatum* were smaller. The reverse of these results was recorded by Camloh and Gogala (1991) who showed that, BA addition to the medium slightly inhibited bud growth of *Platycerium bifurcatum.* Kwa, *et al* (1997a) of the various types of plant growth regulators tested, the callus which arose from cells cultured on medium with cytokinin (Kinetin and BA at 10 µM) showed differences in colouration. Two types of soft callus, dark-green and pale-green, were appeared after 28 days. With BA, growth of these callus masses was poor and the callus turned brown after the first subculture. Kinetin at 10 µM was the optimum level for development and proliferation of two types of callus.

Table (2): Effect of MS salt-strength on survival (%) and callus formation (%) by *Platycerium bifurcatum leaf sections* after one and two

subcultures. Leaf sections were cultured *in vitro*, for 4 weeks, on MS-basal medium supplemented with 2,4-D and kinetin at a rate of 1 mg/L^{-1}

MS salt-strength	Survival (%)		Callus formation (%)	
	After one Subculture	After two subcultures	After one subculture	After two subcultures
MS	83.30 a	100.00 a	80.00 a	100.00 a
½ MS	75.00 ab	100.00 a	66.70 a	88.90 b
¼ MS	66.70 b	100.00 a	43.80 b	62.50 d
1/8 MS	66.70 b	100.00 a	50.00 b	75.00 c

Means followed by different letters are significantly different at the 5% level according to L.S.D.

Table (3): Effect of benzyladenine (BA) concentration on GGB (%) formation of *Platycerium bifurcatum* leaf sections after 8 and 16 weeks

BA Conc. (mg/L^{-1})	GGB (%)	
	After 8 weeks	After 16 weeks
0.0 BA	0.0 0 c	0.0 0 c
3.0 BA	58.30 a	83.30 a
5.0 BA	33.30 b	50.00 b

Means followed by different letters are significantly different at the 5% level according to L.S.D.

Data in Table (4_A) indicated that the best auxin concentration for shoot formation was IAA at 5 or 15mg for percentage of leaf explant differentiation from GGB to shoots (100%). The high concentration of IAA or NAA (15 mg/L^{-1}) was recorded the maximum number of shoots 6.1 and 5.9 shoots/culture, respectively. On the other hand, the little number of shoots was obtained with 15 mg/L^{-1} IBA (2.0 shoots/culture).

Hartmann (1987) cultured shoots apex of *Platycerium stemaria* on modified MS with 15 mg/L^{-1} IAA for stage I and II. Omit IAA in stage III,. the author also found that, the excellent results were given.

All cultures formed rooting (100%) with all concentration of auxins but good roots was observed by using 5mg/l IBA. Camloh and Gogala (1991) reported that, the best results were obtained by 6 µM IBA in the medium in a preliminary rooting experiment. Camloh *et al* (1994) found that, the highest number of roots per explant was achieved on the medium supplemented with 9 µM IAA. They added that the percentage of rooted shoots was relatively low, but when 6-9 µM IBA was added to the medium the rooting was enhanced.

IBA was suitable for callus formation. The most effective concentration was 15 mg/l which produced the heighest percentage of callus (100%).This result agreed with Camloh and Gogala (1991) who observed that, IBA stimulated callus growth of *Platycerium*. Conversely, Camloha *et al* (1994) found that, the amount of callus production increased by increasing NAA concentration.

Table (4_A): Effect of auxin (IAA, IBA and NAA) concentration on growth and development of *Platycerium bifurcatum* GGB explants. GGB explants were cultured *in vitro*, for 4 weeks.

Auxin		Shooting (%)	Shoot number/ culture	Callus formation (%)
Type	Conc. (mg/L^{-1})			
Control	0.0	60 e	3.3 b	0.0 f
1AA	5	100 a	4.7 b	0.0 f
	10	70 d	3.9 b	85.0 b
	15	100 a	6.1 a	70.0 c
IBA	5	40 g	4.3 b	100.0 a
	10	90 b	4.6 b	86.0 b
	15	50 f	2.0 c	100.0 a
NAA	5	60 e	2.2 c	27.0 e
	10	70 d	2.3 c	36.0 d
	15	80 c	5.9 a	88.0 b

Means followed by different letters are significantly different at the 5% level according to L.S.D.

The effect of lower concentrations of NAA (0.0, 0.1, 0.3, 0.5, 0.7 and 0.9 mg/L^{-1}) on *Platycerium bifurcatum* shoots differentiation using GGB segments as explants (0.5 cm) was shown in Table (4_B). Percentage of shoooting was 100% with all concentration of NAA. The number of shoots and leaves were significantly increased by adding 0.9 mg/L^{-1} NAA (50.1 shoots / culture; 131.8 leaves/ culture) respectively and the addition of 0.1 NAA reduced the rate of shoot production (7.3 shoots/ culture), on the surface of the pieces of GGB (green globular bodies) *Platycerium bifurcatum*. The best result was obtained with the culture medium containing 0.1 mg/L^{-1} Kinetin and NAA (Jambor-Benczur *et al,* 1994). Wei (1998) found that, the highest number of globular green bodies (GGB) of *Nephrolepis cordifolia* was produced on MS medium supplemented with 0.05 mg/L^{-1} NAA and 0.05 mg/l IAA. GGB easily regenerated plants on the same medium. Data recorded that, when the GGB segments of *Platycerium bifurcatum* were cultured on growth regulators free medium, shoot production was inhibited (2.0 shoots / culture). Control medium showed a tendency to increase the rate of callus formation and high decrease of roots and with addition of any concentration from NAA inverse this results was observed.

Table (4_B) : Effect of α- naphthalene-acetic acid (NAA) concentration on growth and development of *Platycerium bifurcatum* GGB explants.GGB explants were cultured *in vitro*, for 4 weeks.

NAA Conc. (mg/L^{-1})	Shoots number / culture	leaves number / culture
0.0	2.0 e	0.0 e
0.1	7.3 d	66.5 c
0.3	35.3 b	108.3 b
0.5	29.9 b	84.0 cd
0.7	20.4 c	91.2 bd
0.9	50.1 a	131.8 a

Means followed by different letters are significantly different at the 5% level according to L.S.D.

Kwa *et al.* (1997a), On *Platycerium coronarium,* the presence of NAA in the medium caused profuse rhizoid formation. In control cultures, the cells developed small amounts of callus. Wei (1998), on *Nephrolepis cordifolia* showed that, adventitious root formation was the best on medium supplemented with 0.1 mg/L^{-1} NAA.

Effect of different degree of pH combined with various concentrations of agar on average number of shoots was recorded in Table (5).

Table (5): Effect of agar concentration and pH value on growth and development of *Platycerium bifurcatum* GGB explants. GGB explants were cultured *in vitro*, for 8 weeks, on MS-basal medium supplemented with NAA (0.9 mg/L^{-1}).

Agar conc. (g/l)	pH				
	4.2	4.7	5.2	5.7	6.2
	Shoots number / culture				
0.0	0.0 k	0.0 k	0.0 k	0.0 k	0.0 k
3.0	69.8 c	35.0 j	124.8 a	43.7 ghi	63.8 cd
5.0	47.4 fgh	36.8 ij	118.3 a	47.8 fg	69.2 c
7.0	0.0 k	0.0 k	81.6 b	46.3 gh	54.7 ef
9.0	45.4 gh	0.0 k	59.9 de	54.6 ef	40.2 hij
	Leaves number / culture				
0.0	0.0 k	0.0k	0.0 k	0.0 k	0.0 k
3.0	209.8 c	105.7 j	364.4 a	138.1 g	190.7 d
5.0	143.2 fg	110.7 ij	355.9 a	143.9 fg	207.3 c
7.0	0.0 k	0.0 k	244.8 b	140.0 g	158.3 ef
9.0	137.3 gh	0.0 k	180.3 d	164.7 e	122.4 hi
	Growth value / culture				
0.0	1.3 i	0.8 i	2.4 i	1.2 I	1.4 i
3.0	22.9 cd	11.6 gh	41.5 a	14.1 fgh	25.2 bc
5.0	15.8 efg	11.9 gh	42.9 a	15.4 efgh	26.3 bc
7.0	14.7 fgh	11.5 gh	28.8 b	16.6 ef	21.8 cd
9.0	15.5 efgh	11.2 h	19.5 de	19.5 de	13.9 fgh

Means followed by different letters are significantly different at the 5% level according to L.S.D.

Data showed that the best shoot number (124.8 shoot/ culture) was produced on Murashige and Skoog medium supplemented with 3 gm/L^{-1} agar and no significant difference between this medium and MS medium was solidified by 5 gm/L^{-1} agar (118.3 shoot/culture). The pH value of both media was adjusted at 5.2. The lowest shoot number was found with MS medium + 3 gm/l agar and pH value 4.7. No shoots were appeared when the explants were cultured on liquid medium at different degrees of pH. Similar results were recorded by Higuchi and Amaki (1989) who found that, GGB of *Asplenium nidus* grew more rapidly on agar medium than in liquid medium and the reverse was obtained on *Platycerium bifurcatum* when explants were grown on filter paper bridges on liquid medium (Camloh and Gogala, 1992). Data showed that, the optimum degree of pH was 5.2 for producing the large number of leaves correleated with solidified medium by agar at 3g/L^{-1} (364.4 leaves/culture) or 5 g/L^{-1} (355.9 leaves/culture) and no significant difference between two media was noticed. Lower number of leaves proliferation was achieved on MS medium adjusted to pH 4.7 and solidified by 3.0 or 5.0 gm/l

agar (105.7 and 110.7 leaves/culture, respectively). Also, data in table (4) showed that no leaves were initiated on MS medium solidified by 7.0 or 9.0 gm agar and also no leaves were appeared by using liquid media at all degrees of pH (control medium).

The growth value rates of initial explants were severely reduced in liquid media. The incorporation of agar into the MS basal medium at 3 or 5 gm/l resulted in the maximum rates of growth value especially at pH 5.2, whereas increasing agar level to 7 or 9 gm/L^{-1} resulted in inhibitory effect on growth value especially at pH 4.7. These results are shown in table (5).

Gulsen and Dunanoglu (1991) on *Cydonia oblonga* Mill. showed that, medium pH of 5.5 and 5.7 gave increased shoots number as well as shoot thickness and length. $5g/L^{-1}$ agar gave the best result in shoot number, shoot thickness and shoot length. Also, the rate of shoot multiplication and elongation decreased with increasing agar concentration. The optimum pH for *in vitro* growth of *Nematanthus* was in the range of 5.5 to 5.8. The lower pH values than 5.5 could retared the induction of shoot development on initial explants, whereas increasing pH to higher than 6 significantly decreased the multiplication rate per subculture (Le, 1992).

CONCLUSION

Platycerium bifurcatum plantlets produced by using leaf sections (0.5 cm) when cultured on MS salts + 1mg/l 2,4-D + kinetin for callus induction through three subcultures (4weeks) in Fig 1-a. explants containing callus were transferred to MS medium supplemented with 3mg/l BA (this concentration was fit than 5mg/l BA (Fig 1-b)) for GGB initiation after two subcultures. Fewer shoots were produced after two subcultures on GGB explants when cultured on medium containing higher concentration (5,10 and 15 Is mg/l) of auxines (IAA, NAA or IBA) (Fig 1-c). NAA at concentration lower than 1mg/l was suitable for GGB explants regeneration to shoots and the optimum concentration for the production of maximum shoot numbers was 0.9 mg/l NAA (Fig 1-d).

Plantlets were transferred to greenhouse for acclimatization stage in peatmoss-sand mixture (3:1v/v) (Fig 1-e&f). PH and agar concentration in the medium were effective on growth value of *Platycerium bifurcatum.* The maximum growth value was found with PH 5.2 in MS medium + 0.9 mg/l NAA and solidified medium containing 3mg/l agar (Fig 2).

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حث تكوين الأجنة خضرياً لنباتات البلاتثيرم باستخدام تقنيات الزراعة النسيجية

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جددت بنجاح تكوين نبتات البلاتيثيرم من قطاعات الأوراق من خلال تكوين الكالس وإنتاج الأجسام الكروية الخضراء كمرحلة وسطية ثم تكشفها لإنتاج الأفرع .

تم تحفيز تكوين الكالس الهش من قطاعات الأوراق على بيئة بها أملاح موراشيجى وسكوج (1962) كاملة التركيز مضافا إليها 1 ملجرام 4.2 كلوروفينوكسى حامض الخليك / لتر و1ملجرام كينيتين / لتر خلال عمليتين إعادة زراعة وكل عملية بعد أربع أسابيع. أعلى معدل إنتاج للأجسام الكروية الخضراء حصل عليها بإضافة 3 ملليجرام/ لتر بنزيل أدنين إلى بيئة موراشيجى وسكوج (1962).

اكبر عدد من الأفرع تجدد من القطاعات المحتوية على للأجسام الكروية الخضراء المنزرعة على بيئة موراشيجى وسكوج المحتوية على تركيز نسبيا قليل من نفتالين حامض الخليك (صفر إلي0.9 ملليجرام/لتر) عن عدد الأفرع المتجددة من القطاعات المحتوية على الأجسام الكروية الخضراء المنزرعة على بيئة موراشيجى وسكوج المحتوية على تركيز نسبيا أعلى من ألا وكسينات نفتالين حامض الخليك وحامض اندول بيوتيريك و اندول حامض الخليك (5، 10، 15 ملليجرام/لتر).

أنتج أعلى عدد من الأفرع المحتوية على الأجسام الكروية الخضراء عندما زرعت على بيئة تحتوى على0.9 ملليجرام/لتر نفتا لين حامض الخليك و أعلى معدل نمو و عدد للأفرع لوحظ على القطاعات لمحتوية على الأجسام الكروية الخضراء باستعمال بيئة تحتوى على 0.9 ملليجرام/لتر نفتا لين حامض الخليك و متصلبة بـ 3 جرام /لتر أجار وقيمة درجة الحموضة 5.2 عندما اختبر تأثير تركيزات الاجار ودرجات الحموضة. نمت الأفرع خلال عملية الأقلمة في الصوبة إلي نبتات و كانت الأفرع قد زرعت في آواني بلاستيكية محتوية على بيت موس - رمل(1-3 حجم/حجم).

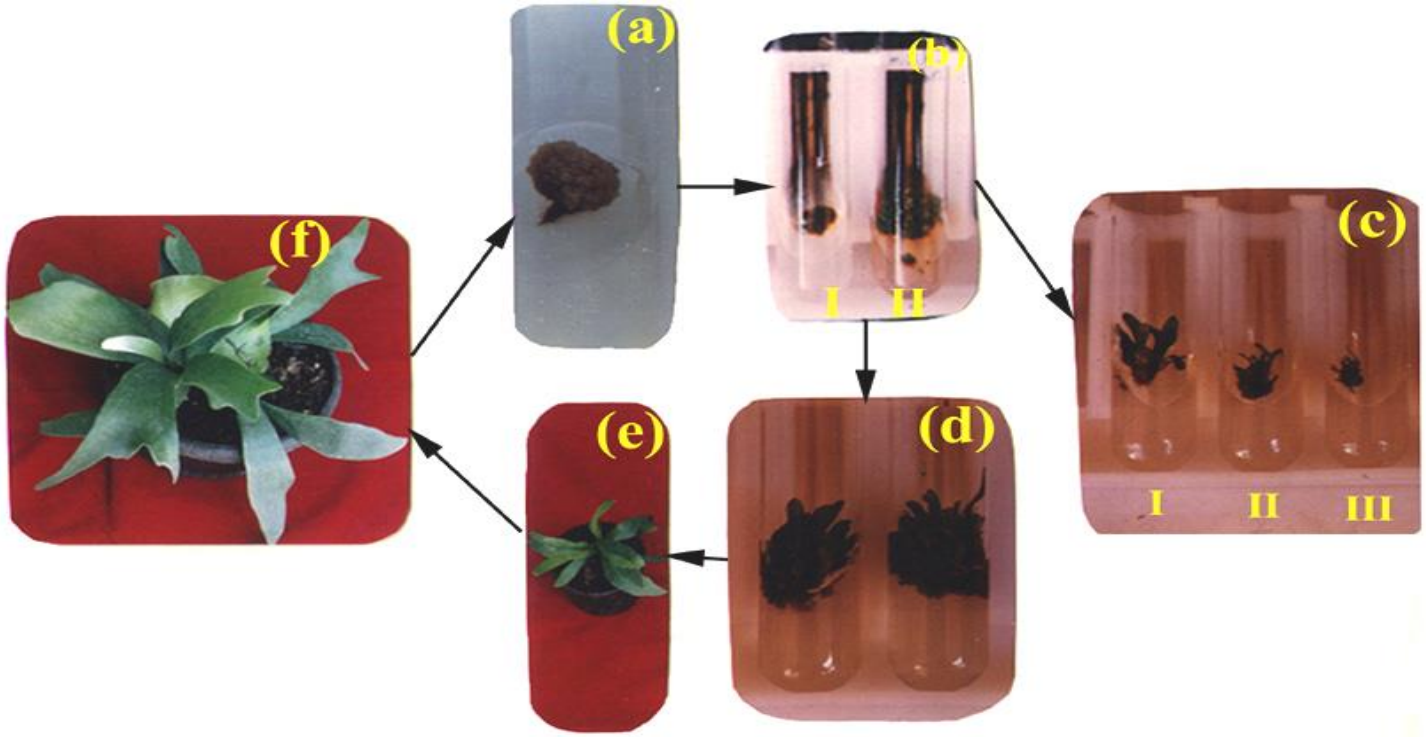

Figure (1):Ascheme of plantlets production from *Platycerium bifurcatum* leaf sections *in vitro* . (a) Friable callus induction on MS salts + 1 mg/L^{-1} 2.4-D + 1 mg/L^{-1} Kinetin. (b) Difference formation between 5 mg/ L^{-1} BA (I) and 3 mg/L^{-1} BA (II) to GGB from callus. (c) Number of shoots regeneration on (I) 15 mg/L^{-1} IAA (II) 15 mg/ L^{-1} NAA (III) 15 mg/L^{-1} IBA. (d) The suitable concentration of NAA (0.9 mg/ L^{-1}) on shoots formation from GGB explants. (e) The establishment in greenhouse after 3 months. (f)After 12 months.