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MICROPROPAGATION AND SUITABLE PROTOCOL TO OVERCOME HYPERHYDRICITY OF *Justicia brandegeana*

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ABSTRACT

For micropropagation of *Justicia brandegeana*, shoot tips were excised from mature plants grown in the greenhouse. These explants were cultured on MS medium containing combination of BA and NAA. Hyperhydricity was a serious problem during the micropropagation of *Justicia brandegeana*. The effects of different factors for overcoming of hyperhydricity were studied. This phenomenon was influenced by level and type of cytokinin, salts media, vessel aeration and agar concentrations. High BA concentrations (2.0 and 3.0 mg/L) gave multiple shoots but led to high incidence of hyperhydricity. WPM was significantly effective in controlling hyperhydricity in shrimp plant compared with MS medium. The treatment of 8.0g/L agar with opening jars (weekly) gave the lowest hyperhydricity percentage (8.39%) without affecting shoot multiplication (11.20 shoot/explant).

The developed shoots were transferred to half- MS medium for rooting. Two types of auxin were tested; IBA or NAA each at 0.0, 0.5 and 1.0 mg/L with or without 3.0 g/L AC. The treatment of 1.0 mg/L NAA and 3.0g/L AC produced the largest number of roots (4.60). The plantlets were transferred from the rooting medium and cultured in 1:1 peatmoss: sand mixture. The survival percentage was 73% after 5 weeks from transplanting.

INTRODUCTION

The shrimp plant, *Justicia brandegeana* (*Beloperone guttata*), family Acanthaceae is an evergreen perennial shrub with spindly limbs, oval green leaves, and white flowers extending from red bracts which look a bit like shrimp...hence the shrub's common name. The bush grows to four or five feet tall, the leaves are oval usually about 2.5 inches long. It is a common ornamental shrub and does best in well-drained sandy or loamy soil, but is generally low maintenance and drought- tolerant. The flowers fade somewhat in the full sun. Shrimp plant can be used as Border, Mass Planting, Specimen, Container Plant and Naturalizing. (Davison, 2001). In Egypt, no satisfactory results were obtained concerning propagation of *Justicia brandegeana* from seed or cuttings (many orcharest). Problems associated with conventional propagation, including poor rooting from cuttings, low seed production have promoted the use of micropropagation as a method to rapid production of many clones. Micropropagation of ornamental plants through tissue culture is very important to produce large number of genetically, healthy, uniform plants. (Al-Maary *et al.*, 1995). *In vitro* propagation presents a very important alternative, because it has higher rates of multiplying clean planting material and the small amount of space required to multiply large number of plants. In our preliminary experiments using shoot tips of *Justicia brandegeana* indicated high incidence of hyperhydricity. However,

micropropagation of *Justicia brandegeana* is hammered by the phenomenon of hyperhydricity (vitrification).

Hyperhydricity is a frequency problem in tissue cultures limiting the growth and multiplication *in vitro* and establishment *ex vitro* (Debergh *et al.*, 1992). It's a serious problem during *in vitro* culture of plants, which directly affects the production at commercial level. This phenomenon is characterized by a "glassy" or " swollen" appearance to the tissue and can often reduce multiplication rates, induce poor quality shoots and ultimately lead to tissue necrosis (Ziv, 1991). Methods employed to controlled hyperhydricity have included improved vessel aeration (Rosetto *et al.*, 1992), reducing cytokinin levels and changing the concentration of medium constituents (Ziv, 1991), increasing agar concentration (Lapena *et al.*, 1992)...etc. Although these modifications can often alleviate symptoms, they can also cause a simultaneous decrease in multiplication rates (Zimmerman *et al.*, 1995).

The objective of this investigation is to identify an efficient and reproducible protocol for shoot proliferation without hyperhydricity for micropropagation of *Justicia brandegeana* from shoot tips and establish the requirements for *in vitro* rooting and acclimatization of plantlets in soil for producing abundant material.

MATERIALS AND METHODS

This study was carried out at Plant Biotechnology Department, Institute of Genetic Engineering and Biotechnology, Minufiya University, during the years of 2004 and 2005.

First Experiment

The aim of this experiment was to determine the optimal combination of Naphthalene acetic acid (NAA) and Benzyladenine (BA) for multiplication from shoot tip culture. Shoot tips were excised (about 7-8 mm) from mature plants grown in greenhouse and were washed with running tap water for 30 min. Surface disinfection of explants was achieved by immersion with 20% commercial bleach solution (v/v) (0.5% sodium hypochlorite) for 10 min. followed by four rinses in sterile distilled water to remove all traces of the disinfectant. The external parts of explants were removed and remained portion was of 0.4- 0.5 cm in length. These explants were cultured on MS (Murashige and Skoog, 1962) medium. This medium was used at full strength supplemented with different concentrations and combinations of NAA and BA.

Aseptic shoot tip explants (0.4 - 0.5 cm) were cultured on MS medium containing 30 g/L sucrose and solidified with 7.0 g/L agar. For shoot proliferation, this medium was supplemented with NAA at 0.0, 0.1 and 0.5 mg/L corresponding to BA at 0.0, 0.5, 1.0, 2.0 and 3.0 mg/L in different combinations. The pH medium was adjusted to 5.7 prior to the addition of agar. Medium was distributed into culture jars (325 ml), each jar contained 40 ml of the medium. The jars were capped with polypropen closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressures for 20 min. All cultures

were incubated at 25±2°C under 16 h using cool white fluorescent light. The following data were collected after 5 weeks from culture initiation:
- Number of shoots per explant.
- Number of hyperhydrified shoots and Hyperhydricity percentage (%) was calculated as:

$$\%\ \text{Hyperhydricity} = \frac{\text{Number of hyperhydrified shoots}}{\text{Number of shoots per explant}} x100$$

Second Experiment

The aim of this experiment was to study the effect of salt formulation media and cytokinins and their concentrations on hyperhydricity of shoots. Aseptic shoot tip explants (as mentioned in the first experiment) were cultured on the surface of the solidified of two type of salt media MS or WPM (Lloyd and McCown, 1981) including two type of cytokinins, BA or Kin each at 0.0, 1.0. 1.5. 2.0 and 3.0 mg/L. The media were gelled with 7.0 g/L agar and 30 g/L sucrose. The pH medium was adjusted to 5.8 prior to the addition of agar. The media were distributed into culture jars (325 ml), each jar contained 40 ml. Jars were capped with polypropene closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressure for 20 min. All cultures were incubated at 25± 2°C under 16 h using cool white fluorescent light. Data collected after 5 weeks of cultures were number of shoots, number of hyperhydrified shoots and shoot height.

Third Experiment

The aim of this experiment was to study the effect of opening cultured jars for about 7 minutes (as a method for air exchange) and agar concentrations on the shoot multiplications as well as on the hyperhydricity of *Justicia brandegeana*.

The best type and concentration of cytokinin (2.0 mg/L BA) and salt medium (WPM) were selected from previous experiment to test the effect of agar concentrations and opening culture jars on multiplication and hyperhydricity.

Aseptic shoot tips were cultured on WPM supplemented with 2.0 mg/L BA and agar at five concentrations (6.0, 7.0, 8.0, 9.0 and 10.0 g/L) combining with opening the culture jars in lamina flow (hood) every 7 days (for changing air in jars) or without opening for 5 weeks. The medium was distributed into culture jars. The jars were capped with polypropene closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressure for 20 min. Three explants were cultured per jar and each treatment included five replicates. All cultures were incubated at 25±2°C under 16 h using cool white fluorescent light. The following data were recorded after 5 weeks:

-Number of shoots per explant
-Number of hyperhydrified shoots

Statistical Analysis

Experiments were set up in completely randomized block design with five replicates; each replicate consisted of one jar containing three explants. All experiments were repeated two times. The results were analyzed by analysis of variance (ANOVA) according to Gomez and Gomez (1984).

Rooting and Acclimatization

Proliferated and non-hyperhydrified shoots were individually separated (about 1 cm in length) and cultured on rooting medium. The medium was half strength MS salts supplemented with myo-inositol at 100 mg/L, sucrose at 30 g/L. For rooting, two types of auxins were tested; IBA or NAA at 0.0, 0.5 and 1.0 mg/L with or without 3.0 g/L activated charcoal (AC). The pH medium was adjusted to 5.8 prior to the addition of 7.0 g/L agar. The medium was distributed into culture jars. The jars were capped with polypropene closures and autoclaved at 121°C and 1.2 kg/cm^3 air pressure for 20 min. Three shoots were cultured per jar and each treatment included five replicates. The culture jars were kept under the same environmental conditions as described with previous experiment. After 4 weeks, number of roots /shoot was recorded.

The shoots with well-developed roots were washed with tap water to remove agar and transferred to mixture of 1:1 v/v (peatmoss and sand) in small plastic pots. The pots were maintained in a greenhouse and a solution of half strength MS salts was added to the pots to develop plants. After 4 weeks, the survival percentage was recorded.

RESULTS AND DISCUSSION

Effect of BA and NAA and their combination on multiplication and hyperhydricity.

Table (1) and Fig (1) show the effect of BA and NAA and their interaction on the number of both shoots per explant and hyperhydrified shoots and percentage of hyperhydricity. Data of the main effect of BA on the number of shoots per explant (multiplication) show that shoot multiplication was gradually increased with increasing BA concentrations up to 2.0 mg/L , then a little decrease (9.13) in this parameter was noticed at 3.0 mg/L BA compared with 2.0 mg/L which recorded the highest recorded (10.86).

Table (1): Effect of BA and NAA concentrations on shoot proliferation and hyperhydricity from shoot tip culture of *Justicia brandegeana* after 5 weeks on MS medium.

Treatments (mg/l)	Number of shoots per explant			Mean A	Number of hyperhydrified shoots			Mean A	% hyperhydricity		
NAA (B) / BA(A)	0.0	0.1	0.5		0.0	0.1	0.5		0.0	0.1	0.5
0.0	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00
0.5	6.60	6.20	6.20	**6.33**	1.46	2.80	4.00	**2.75**	22.12	45.16	64.52
1.0	9.80	9.40	6.80	**8.66**	6.00	6.60	5.20	**5.93**	61.22	70.21	76.47
2.0	12.80	10.80	9.00	**10.86**	9.60	8.20	7.40	**8.40**	64.06	68.52	82.22
3.0	9.20	8.80	9.40	**9.13**	7.60	8.20	9.20	**8.33**	82.61	93.18	97.87
Mean B	**7.68**	**7.04**	**6.28**		**4.93**	**5.16**	**5.16**				
LSD 5%	**A = 0.80 B = 0.62 AxB = 1.39**				**A = 0.62 B = NS AxB = 1.08**						

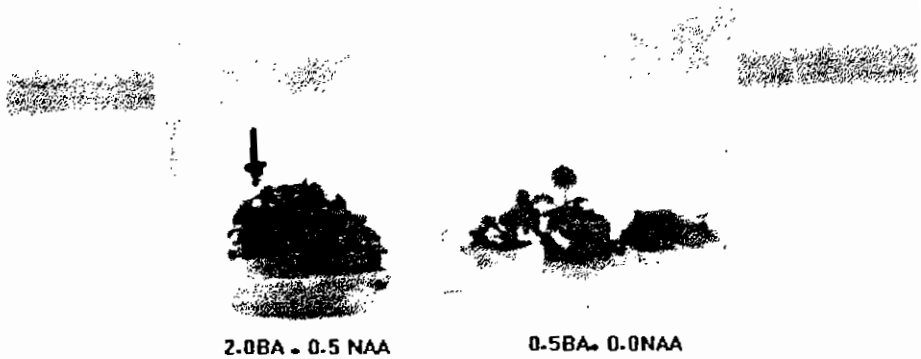

Fig (1): Multiplication rate from shoot tips cultured on MS medium and hyperhydrified shoots (arrow) on MS medium after 5 weeks from culturing *in vitro*.

On the contrary, increasing NAA levels from 0.0 to 0.5 mg/L caused a gradual decrease in this parameter and the highest level (0.5 mg/L) was more pronounced (6.28). Effect of the interaction between BA and NAA on the number of shoots per explant indicates that no shoots were proliferated in the absence of either BA and NAA or BA alone. Data also clear that 2.0 mg/L BA alone gave the highest record (12.80) followed by 2.0 mg/L BA with 1.0 mg/L NAA (10.80), while the lowest value (6.20) was obtained with the media contained 0.5 mg/L BA combined with either 0.1 or 0.5 mg/L NAA. In several plant species, combinations of cytokinins and auxins are reported to stimulate the *in vitro* multiplication of shoots. The plant growth regulators, BA and NAA are often used for the induction of shoots (Tripepi, 1997). In this regard when IBA was included in the culture medium at 0.1 or 0.5 mg/L concentrations, the higher BAP levels (2.0 or 3.0 mg/L) became less effective for shoot formation of *Amygdalus communis* (Gürel and Gülsen, 1998).

Concerning the effect on the number of hyperhydrified shoots the same Table reveal that increasing BA levels increased hyperhydricity of shoots gradually. The highest record (8.40 that act as 77.35% from number of shoots per explant) was obtained at 2.0 mg/L BA. Maghazy Eman *et al.*, (2002) indicated that increasing levels of BA in culture medium increased the occurrence of vitrification phenomenon of *Dianthus caryophyllus*. Moreoever, incidence of hyperhydricity was high in all combinations of growth regulators. Data clearly show that presence of auxin combined with cytokinin increased hyperhydricity up to 9.20 and 97.87 % for number of hyperhydrified shoots and hyperhydricity percentage respectively. These results are in agree with that of Debergh *et al.*, (1992) who reported that hyperhydricity was more strongly linked to the presence of auxin and to the auxin/cytokinin balance, than to the cytokinin level or the number of subcultures.

Data in Table (1) clear that hyperhydricity percentage was gradually increased with increasing both BA and NAA together in the media. A possible explanation could be that the auxin/cytokinin balance of regeneration media affected the DNA level of regenerates. In turn, an abnormal DNA content was systemically associated with severe hyperhydricity symptoms (Ochatt *et al.*, 2002). The highest percentage (97.87%) was obtained with 3.0 mg/L BA combined with 5.0 mg/L NAA, while the lowest one (22.12%) was obtained with 0.5 mg/L BA alone.

Effect of cytokinin types (BA and Kin) at various concentrations and culture media salts on multiplication.

Data in Table (2) show the effect of two types of cytokinin (BA and Kin) at various concentrations and culture media salts on the number of shoots per explant (multiplication) after 5 weeks from culture. Data clear that insignificant effect between MS and WPM media was observed on shoot multiplication. Data also clear that BA was more effective than Kin in increasing this character. Shoot number was significantly increased with increasing cytokinin concentrations. The highest shoot number (11.40) was obtained by 2.0 mg/L cytokinin compared with control or other levels. Similar result was obtained by Chan *et al.*, (2003) who reported that basic MS medium supplemented with 2.0 mg/L BA was found to be the most suitable medium for induction of multiple shoots in *Caladium hortulanum* and *Caladium bicolor*. Concerning the interactions between culture media and cytokinin (types or concentrations) insignificant effects on this parameter were observed.

Table (2): Effect of different types of cytokinin at various concentrations and culture media salts on the number of shoots/explant of *Justicia brandegeana* after 5 weeks *in vitro*.

		Concentration of cytokinin (C)					Mean (A x B)	Mean of (A)
Media (A)	Cytokinin type(B)	0.0	1.0	1.5	2.0	3.0		
MS	BA	0.00	9.60	10.80	13.20	9.00	**8.52**	**7.76**
	Kin	0.00	6.40	8.40	10.00	10.20	**7.00**	
WPM	BA	0.00	9.20	10.40	12.20	10.40	**8.44**	**7.64**
	Kin	0.00	6.60	7.80	10.20	9.60	**6.84**	
Mean of (C)		**0.00**	**7.95**	**9.35**	**11.40**	**9.80**	**Mean of (B)**	
Mean of (B x C)	BA	0.00	9.40	10.60	12.70	9.70	**8.48**	
	Kin	0.00	6.50	8.10	10.10	9.90	**6.92**	
Mean of (A x C)	MS	0.00	8.00	9.60	11.60	9.60		
	WPM	0.00	7.90	9.10	11.20	10.00		
LSD at 5%		**A = NS** **B = 0.46** **C = 0.74** **A x B = NS** **A x C = NS** **B x C = 1.04** **A x B x C = NS**						

However, a significant increase was obtained with increasing either BA or Kin concentrations compared with the control. The highest values in this respect (12.70 and 10.10) were obtained by using 2.0 mg/L for both BA and Kin, respectively. Regarding the interaction effect among cytokinin type and concentration and culture media, data in the same Table reveal that no shoots were proliferated in the absence of hormones in MS or WPM media. Also, the highest records were obtained with BA at 2.0 mg/L for both MS and WPM media (13.20 and 12.20, respectively). However, the lowest records were obtained with Kin at 1.0 mg/L for both MS and WPM media (6.40 and 6.60 respectively), but these results did not reach to the significant level at 5%.

Effect of type of cytokinin and their concentration and culture media on hyperhydricity.

Data in Table (3) reveal the main effect of culture media on the number of hyperhydrified shoots. It clearly shows that WPM (4.38) was significantly effective in controlling hyperhydricity in shrimp plant compared with MS medium (6.00).Similar results were reported by Thomas *et al.*,(2000) who found that Rugini Olive (RO) basal medium showed better growth and less hyperhydricity than MS medium. That may be due to low concentration of mineral, especially ammonium in WPM than MS medium. A low nitrogen concentration medium, such as a half-strength of MS or a WPM media is a more suitable mineral combination than full-strength MS. Data of the same Table clear that although number of hyperhydrified shoots was significantly decreased by Kin (4.32) than BA (5.46) but, Kin was more effective than BA in increasing hyperhydricity, since hyperhydricity percentage of Kin was 71.10% compared with that of BA (64.39%). Kadota and Niimi (2003) showed that hyperhydricity in explants of *Pyrus pyrifolia* is affected by type of cytokinin, but the concentration has little influence. The difference observed in susceptibility to hyperhydricity depended on the type of cytokinin used (Ochatt *et al.*, 2000). In addition, number of hyperhydrified shoots was gradually decreased with decreasing cytokinin level up to 1.0 mg/L. Although several factors are generally involved in the induction or development of shoot hyperhydricity, an excess of cytokinins has been reported to be a major factor involved in shoot hyperhydricity during *in vitro* propagation of several plant species (Kataeva *et al.*, 1991). Concerning the interaction effects, Table (3) indicates that there was insignificant effect on the number of hyperhydrified shoots between cytokinin type and either culture media or cytokinin levels. Whereas, the interaction effect between culture media and cytokinin concentrations had a significant effect on this parameter. Data clear that this parameter was restricted by decreasing cytokinin levels up to 1.0 mg/L (5.10 and 3.40 for MS and WPM media, respectively). Regard to the interaction among culture media and cytokinin types and concentrations, the same data indicate that insignificant effect on this character was observed. Generally in both culture media the number of hyperhydrified shoots was increased with increasing cytokinin under this study, especially MS medium supplemented with BA.

It is of important to conclude the hyperhydricity percentage which can be calculated from Tables (2 and 3) mentioned previously. The concentrations of cytokinin, types of cytokinin and culture medium and their interactions affected hyperhydricity percentage in shrimp plant. Accordingly, hyperhydricity percentage ranged from 42.42% in the treatment of WPM supplemented with 1.0 mg/L Kin to 93.33% in the treatment of MS supplemented with 3.0 mg/L BA. Although the treatment of WPM with 1.0mg/L Kin gave the lowest percentage, but a lower number of shoots per explant (6.60) also was obtained. Therefore, the treatment of WPM with 2.0mg/L BA was selected to continue the next experiment, since it gave a considerable number of shoots (12.20) and a lower hyperhydricity percentage (47.54).

Table (3): Effect of different types of cytokinin at various concentrations and culture media salts on the number of hyperhydrified shoots of *Justicia brandegeana* after 5 weeks *in vitro*.

		Concentration of cytokinin (C)					Mean (A x B)	Mean of (A)
Media (A)	Cytokinin type(B)	0.0	1.0	1.5	2.0	3.0		
MS	BA	0.00	5.60	7.60	10.20	8.40	6.36	6.00
	Kin	0.00	4.60	6.40	8.40	8.80	5.64	
WPM	BA	0.00	4.00	5.20	5.80	7.80	4.56	4.38
	Kin	0.00	2.80	5.00	5.60	7.60	4.20	
Mean of (C)		0.00	4.25	6.05	7.50	8.15	Mean of (B)	
Mean of (B x C)	BA	0.00	4.80	6.40	8.00	8.10	5.46	
	Kin	0.00	3.70	5.70	7.00	8.20	4.92	
Means of (A x C)	MS	0.00	5.10	7.00	9.30	8.60		
	WPM	0.00	3.40	5.10	5.70	7.70		
LSD at 5%		A = 0.36 B = 0.36 C = 0.57 A x B = NS A x C = 0.81 B x C = NS A x B x C =NS						

Effect of agar concentrations and air exchange on multiplication and hyperhydricity.

The number of shoots per explant, the number of hyperhydrified shoots and hyperhydricity percentage as affected by agar concentration and opening culture jars (without subculture) are shown in Table (4). Data reveal that the shoot multiplication was significantly decreased with increasing agar concentrations from 6 to 10 g/L, except at 7.0g/L, since an insignificant increase was observed (13.70) compared with the treatment of 6.0 g/L agar (13.00). As a conclusion, increasing agar concentrations was more effective in controlling hyperhydricity and producing normal shoots. However, increasing agar had a negative effect on multiplication rate of *Justicia brandegeana*. This is consistent with a reduction in BA uptake by the explants

at higher agar concentration as found by Bornman and Vogelmann (1984). Growth and shoot proliferation decreased with increasing agar amount (Manoj *et al.*, 2003). However, opening culture jars resulted in a significant increase shoot multiplication (10.80) compared with the closed one (9.72). Fal *et al.*, (1999) reported that longer shoots of *Dianthus caryophyllus* were produced when the rate of gas exchange in the culture vessel was increased by using vented closures, which also prevented hyperhydricity and increased the multiplication coefficient in cultures. Beruto and Portogallo, (2000) found that improving air-exchange during the culture period without renewing the medium improved shoot elongation and allowed a better leaf development. Furthermore, insignificant effect was noticed due to the interaction between agar concentrations and opening culture jars on shoots number (Table, 4).

Table (4): Effect of opening culture jars and agar concentrations on shoot multiplication and hyperhydricity of *Justicia brandegeana* after 5 weeks from shoot tip culturing on WPM.

Treatments	Number of shoots per explant		Mean A	Number of hyperhydrified shoot		Mean A	% hyperhydricity	
opening (B) / Agar g/L(A)	Without opening	opening		Without opening	opening		Without opening	opening
6.0	12.60	13.20	**13.00**	9.40	7.20	**8.30**	73.44	54.55
7.0	12.80	14.60	**13.70**	5.60	1.92	**3.76**	43.75	13.15
8.0	10.00	11.20	**10.60**	4.60	0.94	**2.77**	46.00	8.39
9.0	7.80	8.60	**8.20**	1.14	0.00	**0.57**	14.62	0.00
10.0	5.20	6.40	**5.80**	0.00	0.00	**0.00**	0.00	0.00
Mean B	**9.72**	**10.80**		**4.14**	**2.01**			
LSD 5%	**A =0.88** **B = 0.55** **AxB = NS**			**A =0.97** **B = 0.61** **AxB = 1.38**				

As for number of hyperhydrified shoots, data in the same Table indicate that a gradually significant decrease of hyperhydricity from 8.30 to 0.00 was occurred as a result of the gradual increase in agar concentration up to 10.0g/L. This may be due to less relative humidity in the culture jars. Similar result was obtained by Ziv (1991) who clear that the high relative humidity in the atmosphere of *in vitro* cultures, as well as the water potential in the culture medium, has been considered key factors in hyperhydricity. In the herein work, opening culture jars weekly decreased hyperhydricity in *Justicia brandegeana*. This may be attributed to gas exchange, which led to evaporate accumulated ethylene and high relative humidity in jars atmosphere. These were considered as important factors influencing hyperhydricity. In this concern, Saher *et al.*, (2004) reported that the level of ethylene measured in the hyperhydric cultures was significantly higher compared to the normal shoots in all cultivars. Accumulations of gases such as ethylene and CO_2 have also been found to be responsible for hyperhydricity (DeProft *et al.*, 1985). Hyperhydricity was exacerbated when cultured in airtight culture vessel and high ammonium concentrations in common media formulations and drastically reduced the quality of *in vitro*

cultures (Reyes *et al.*, 2004). Concerning the interaction effect, data reveal that this character was significantly decreased by increasing agar concentration with opening culture jars. In addition, 9.0 g/L agar with opening jars and 10.0 g/L agar with or without opening jars prevented hyperhydricity.

Hyperhydricity percentage shown in the same Table indicates that this percentage was decreased from 73.44 to 0.00% due to increasing agar concentration in the media, especially with opening culture jars. The treatment of 8.0g/L agar with opening jars gave the lowest hyperhydricity percentage (8.39%) and a considerable shoot multiplication (11.20).

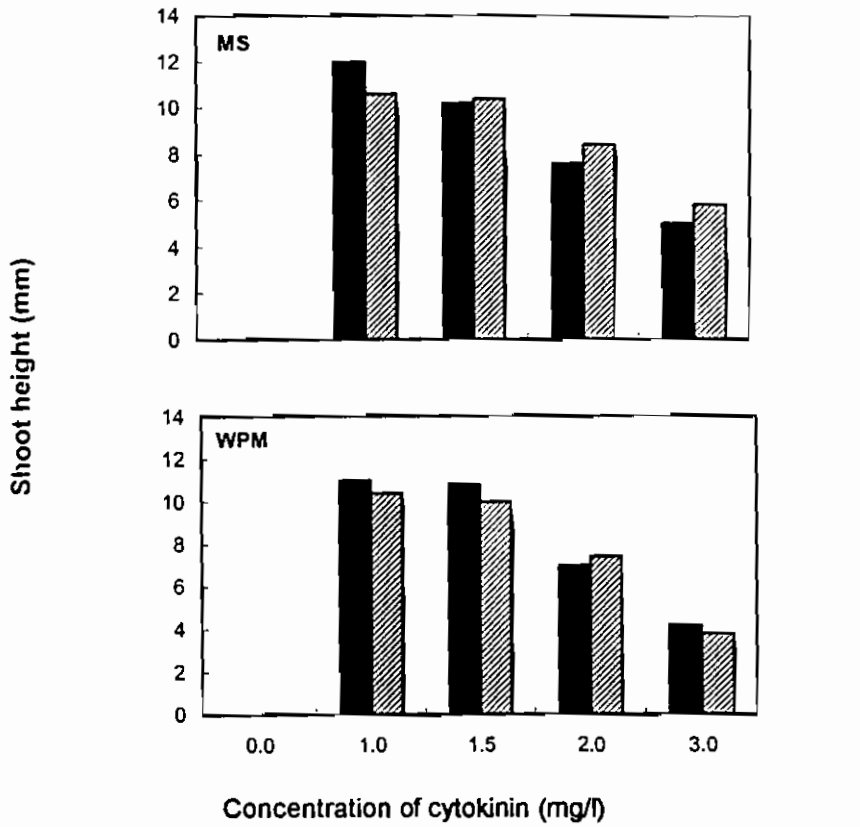

Fig (2): Effect of different types of cytokinin at various concentrations and culture media salts on shoot height of *Justicia brandegeana* after 5 weeks *in vitro*. Pars presented of two cytokinins (BA■ and Kin▨).

Effect of type of cytokinins at various concentrations and culture media salts (MS, WPM) on shoot height.

As for the interaction effect among cytokinin type and concentration and culture media on shoot height, Fig (2) shows that shoot height was decreased with increasing cytokinin level in both MS and WPM media. Although the treatments of 1.0 mg/L BA gave the highest shoot (12.00 and

11.20 for MS and WPM, respectively), while the treatments of 3.0 mg/L gave the lowest records (4.20 and 3.80 for BA and Kin, respectively) only in WPM, but all results did not reach to the significant level at 5%

Effect of AC and auxin type and concentration on rooting.

Successful rooting of the *in vitro* grown shoots is an important factor for establishing tissue culture derived platelets on the soil. For this purpose, the present experiment was carried out for root formation in shoot cultured on half-MS medium.

Data in Table (5) reveal that rooting of microshoots of *Justicia brandegeana* is difficult. Rooting did not occur in microshoots placed on half-MS medium without growth regulators. Presence of AC at 3.0g/L increased the number of roots (2.30) significantly compared with that of 0.00g/L (1.86). Charcoal addition to the medium might act as a darkness agent for promoting adventiticus root formation on proliferated promote rooting (Zimmerman and Broom, 1981). Addition of activated charcoal (AC) to the auxin-containing medium markedly improved rooting behavior of the microcutting and checked callus formation at the microcutting base (Amin, 1992).

In addition, insignificant effect between IBA and NAA on the number of roots was observed. As for the concentrations of auxin, the same Table shows that the number of roots was significantly increased from 0.00 to 3.55 due to the increase in auxin level from 0.0 to 1.0mg/L Fig (3).

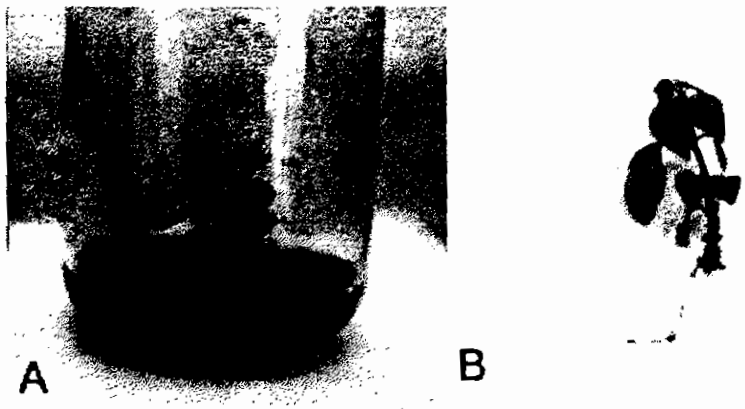

Fig (3): Shoots of *Justicia brandegeana* cultured on half strength MS medium with 1.0 mg/L NAA and 3.0 g/L AC for rooting (A) plantlet ready for planting in pot (B).

Data in the same Table clear that there were insignificant differences between the interactions effect of AC and auxin type and concentration on the number of roots. Regarding the interaction effect among AC and auxin type and concentrations, data reveal that no roots were formed in the absence of IBA or NAA. The highest number of roots (4.60) was obtained with the treatment of 3.0 g/L AC combined with 1.0 mg/L NAA, but these results did not reach to the significant level at 5%. Similar results was obtained by (Çöçü *et al*,. 2004) who reported that on calendula regenerated

shoots were excised and rooted ready within 3 weeks in half-MS medium containing 1.0 mg/L NAA. The rooted plants were transferred to pots containing 1:1 peatmoss and sand mixture, where 73% of plants survived for 5 weeks.

Table (5): Effect of AC and auxin type and concentration on number of root of *Justicia brandegeana* after 4 weeks on hatf strength MS salt medium.

		Concentration of Auxins (C) (mg/L)			Mean (A x B)	Mean of (A)
AC (g/L) (A)	Auxin type(B)	0.0	0.5	1.0		
3.0	IBA	0.00	2.80	3.40	2.06	2.30
	NAA	0.00	3.00	4.60	2.53	
0.0	IBA	0.00	2.40	3.20	1.86	1.86
	NAA	0.00	2.60	3.00	1.86	
Mean of (C)		0.00	2.70	3.55	Mean of (B)	
Mean of (B x C)	IBA	0.00	2.60	3.30	1.96	
	NAA	0.00	2.80	3.80	2.20	
Mean of (A x C)	3.0 g/L	0.00	2.90	4.00		
	0.0 g/L	0.00	2.50	3.10		
LSD at 5%		A = 0.30 B = NS C = 0.36 A x B = NS A x C = NS B x C = NS A x B x C =NS				

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الاكثار الدقيق لنبات الجمبرى ومعالجه ظاهره التزجج

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فصلت القمه الناميه من نباتات ناميه بالصوبه وذلك لاجراء الاكثار الدقيق لنبات الجمبرى وزرعت هذه القمم على بيئه موراشيج وسكوج محتويه على بنزيل ادينين بتركيزات صفر ، ٠,٥ ، ١,٠ ، ٢,٠ و ٣,٠ ملليجرام / لتر مع نفثالين حامض الخليك بتركيزات صفر و ٠,١ و ٠,٥ ملليجرام/ لتر . لوحظت مع مرحله التضاعف ظاهره التزجج وكانت ملازمه لكل المعاملات تقريبا بنسب مختلفه.

درست بعض العوامل المؤثره على هذه الظاهره ومنها بيئات الزراعه (املاح) حيث قورنت بيئه MS و WPM و نوعان من السيتوكينين هما بنزيل ادينين والكاينتين عند تركيزات صفر ، ١,٠ ، ١,٥ ، ٢,٠ و ٣,٠ ملليجرام/لتر وكذلك تهويه اوعيه الزراعه واستخدام تركيزات مختلفه من الاجار (٦ ، ٧ ، ٨ ، ٩ ، ١٠ جم/لتر). اظهرت المعاملات المحتويه على تركيزات مرتفعه من البنزيل ادينين (٢,٠ و ٣,٠ ملليجرام/ لتر) تفوقا من حيث معدل التضاعف ولكنها ادت الى زياده النسبه المئويه لظاهره التزجج.

ادى استخدام املاح بيئه WPM الى تقليل التزجج بشكل واضح عما اذا استخدمت املاح بيئه MS. كانت هذه الظاهره واضحه فى المعاملات المحتويه على البنزيل ادينين عن المحتويه على الكاينتين عند نفس التركيز. اضافه ٨ جم/لتر اجار لم يؤثر بدرجه كبيره على معدل التضاعف ولكن قلل النسبه المئويه للظاهره بوضوح . كذلك فتح اوعيه الزراعه للتهويه كل سبعه ايام وغلقها مره اخرى هام جدا لكل من معدل التضاعف وتقليل هذه الظاهره. اما اضافه ٨ جرام/لتر لبيئه الزراعه مع تهويه اوعيه الزراعه (اسبوعيا) ادى الىظهور اقل نسبه مئويه للتزجج (٨,٣٩ ٪) مع عدم التاثير بدرجه كبيره على معدل التضاعف (١١,٢٠).

لتجذير الافرع الناتجه تمت مقارنه نوعان من الاوكسين هما اندول حامض البيوتيريك ونفثالين حامض الخليك عند تركيزات صفر ، ٠,٥ ، ١,٠ ملليجرام/ لتر مع او بدون ٣ جم/لتر فحم نشط. تفوقت المعامله المحتويه على ١,٠ ملليجرام/لتر نفثالين حامض الخليك مضافا" اليها ٣ جم/لتر فحم نشط حيث اعطت اكبر عدد من الجذور على الفرع (٤,٦). وقد تمت اقلمه النباتات بنجاح عند نقلها للصوبه وزراعتها فى خليط من كميات متساويه حجما من البيتموس و الرمل وكانت نسبه النباتات الحيه بعد خمسه اسابيع من النقل للصوبه هى ٧٣٪.