In vitro propagation of *Hydrangea macrophylla Thunb*

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ABSTRACT

Five experiments were conducted using explants from mature Hydrangea macrophylla plants, with the aim of developing an in vitro propagation protocol for production of multipurpose H. macrophylla plants. The results of exp. 1 indicated that the highest percentage of contamination-free explants (100%) was obtained by using chlorox at 50% plus mercuric chloride (MC) at the concentration 0.2%. Data of exp. 2 indicated that using MS medium plus BA at the rate of 2 mg/l resulted in the highest number of shoots/explant. Increasing the number of subcultures significantly increased the number of shoot per explants, with the 3rd subculture giving the highest value. Shoot length was significantly reduced by adding TDZ to the MS medium. MS medium plus BA or Kin at the rate of 2 mg/l were the most effective treatments in giving significant increases in shoot length. Culturing the explants on an MS medium supplemented with BA at 4 mg/l produced the highest number of leaves/explant. Results of full strength B5 medium was the best medium for causing significant increases in the number of shoot per explant. Increasing the number of subcultures significantly increased the number of shoot per explant. The longest shoots were obtained in the 3rd subculture. Half strength B5 medium was the best treatment for producing the longest shoots in the three subcultures. Using full strength B5 medium led to production of the greatest number of leaves/explant. Results of exp. 4 showed that the greatest number of roots/shootlet recorded with 1/2 MS salt strength medium supplemented with 2 mg IBA/l and activated charcoal. The tallest plantlets were recorded when using MS medium supplemented with 3 mg/l IBA and activated charcoal. The highest number of leaves/shootlet was recorded by using MS medium supplemented with 2 and 3 mg/l IBA. Finally, result of exp. 5 showed that during adaptation, the tallest plants and the greatest number of leaves were observed when using peat moss plus sand (1:1 v/v), where the survival percentage was 100%.

Key words: *Hydrangea macrophylla, micropropagation, BA, kinetin, thidiazuron, IBA, activated charcoal, acclimatization.*

INTRODUCTION

Hydrangeas (*Hydrangea macrophylla* Thunb belonging to the Saxifragaceae family) are potted florists plants and deciduous landscape shrubs. As potted plants, their inflorescences are spectacular spheres of pink, blue or white. They are produced primarily for the Easter season (March- April) and mother's day. The plant is better known internationally as "hortensia" (Bailey and Bailey, 1976). The plants have small-toothed dark green leaves on stiff-erect stems, and medium size flower heads.

Very little has been reported on the micropropagation of *Hydrangea macrophylla*. Sebastian and Heurser (1987) placed dormant buds of *H. quercifolia* on MS medium,

(Murashige and Skoog, 1962) *in vitro* with 10 μM benzyladenine (BA), Zeatin, or 2-isopentenyl adenine (2ip), for the study of leaf callus differentiation and axillary shoot proliferation. They were successful, but published very little data. Therefore, many details are missing in the literature about micropropagation of this species.

According to most sources, seed germination in *Hydrangea spp.* is not difficult (Dirr and Heuser, 1987; Young and Young, 1992; Hill and Hill, 1995); however, the seedlings exhibit variability and do not always produce the desired characteristics or forms (Hartmann *et al.*, 1997). Because of the clonal uniformity that occurs when using cuttings, most of commercial propagation of *Hydrangea macrophylla* is done by root cuttings (Hartmann *et al.*, 1997; Jacobs *et al.*, 1990).

Cytokinins BA, 2iP or zeatin are used for micropropagation of *Hydrangea spp.* I am not aware of any reports on the use of thidiazuron (TDZ) on members of this genus. TDZ is among the most active cytokinin like growth substances used for tissue culture of woody plants species, and at low concentrations it induces axillary shoots proliferation, but may inhibit shoot elongation and at high concentrations promotes formation of callus, adventitious shoots and somatic embryos (Heutteman and Preece, 1993).

The goal of this research was to set a protocol for *in vitro* propagation of *Hydrangea macrophylla* for commercial production. This was done by studying the following points: the effect of sterilization treatments; effect of cytokinins (BA, Kin, and TDZ); effect of different shooting media (MS, WPM, B5, and LS); effect of IBA and activated charcoal on rooting behavior; and effect of growing media during the adaptation stage.

MATERIALS AND METHODS

This investigation was carried out in the Plant Tissue Culture Laboratory in El-Zohreya Botanical Garden, Ministry of Agriculture, Egypt, and Faculty of Agriculture, Cairo University, during the years 2004 and 2005.

Plant material

Shoot tips (1-2 cm), taken from mature plants grown in a greenhouse in El-Zohrya botanical garden, were used in this study. The study included five experiments, as follows.

Experiment (1): Effect of some sterilization treatments on contamination of explants

The aim of this experiment was to study the effect of some sterilization treatments (chlorox solution at concentrations of 30, 40, 50 and 60%, alone or with mercuric chloride (HgCl2) at concentrations of 0.0, 0.1, 0.2, and 0.4%) on the contamination of *Hydrangea macrophylla* explants cultured in vitro. The explants were dipped in ethanol (70%) for 30 sec. before application of the sterilization treatments. Tween 20 (Polyoxyethylene sorbitan monolaurate) was used as a wetting agent at the rate of one drop per 100 ml of the sterilizing solution, in which the explants were dipped for 20 min. (for the different treatments). After sterilizing the explants, they were rinsed in sterilized distilled water (3 times) to remove all traces of the sterilizing substances. All steps of the sterilization procedure were done under aseptic conditions inside the culture cabinet (Laminar airflow) using sterilized instruments.

After the treatments, the explants were cultured in jars on a full strength, hormone-free, MS basal medium. Each jar contained one explant. Plants receiving the different treatments were incubated for one month. After this period, the percentage of contamination-free explants was recorded.

This experiment included 16 treatments replicated 3 times, with each replicate consisting of 10 jars.

Experiment (2): Effect of BA, Kin and TDZ on shooting behavior

Explants of *Hydrangea macrophylla* were cultured *in vitro* on an MS medium supplemented with BA, Kin and TDZ each at concentrations of 0.5, 1.0, 2.0 and 4.0 mg/l, in addition to control explants grown on a hormone-free MS medium. Each treatment (13 treatments) consisted of 4 replicates, with each replicate consisting of 4 jars.

After 4, 8 and 12 weeks (3 subcultures) the following data were recorded: number of shoot/ explant, shoot length (cm), and number of leaves /explants.

Experiment (3): Effect of different media on shooting behaviour

In this experiment, four media were examined: Murashige and Skoog (MS), Woody plant medium (WPM), Gamborg (B5, as described by Gamborg *et al.*, 1968), and Linsmaier and Skoog (LS, as described by Linsmaier and Skoog, 1965). The chemical composition of the four media is shown in Table (A). The tested media were used at full and half strength. After 4, 8 and 12 weeks (representing 3 subcultures) from the date of culturing the explants, the following data were recorded: number of shoot/ explants, shoot length (cm), and number of leaves /explants. In this experiment, the 8 treatments were replicated 4 times, with each replicate consisting of 4 jars.

Experiment (4): Effect of IBA and activated charcoal on rooting behavior and vegetative development.

This experiment was carried out to study the effect of MS (at half salt strength) and different IBA concentrations (1.0, 2.0 and 3.0 mg/l), with or without activated charcoal (A.C.) at 1.0 gm/l, on root formation.

In this experiment, the 8 treatments were replicated 4 times, with each replicate consisting of 4 jars. After 30 days from culturing the explants, the following data were recorded: the number of roots/shootlets, root length (cm), plant height (cm), and number of leaves/shoot.

Experiment (5): Effect of some growing media on acclimatization

This experiment was conducted in the greenhouse to valuate the effect of some growing media on the survival percentage of *Hydrangea macrophylla* plantlets during the acclimatization stage.The plantlets (4-6 cm length with 6-8 leaves) produced *in vitro* were individually transplanted into 8 cm plastic pots filled with: peatmoss (100%), peatmoss + sand (1:1 v/v), or peatmoss + vermiculite (1:1 v/v).

Table (A): Chemical components and media used, (MS, WPM, B5, LS).

Chemical components	Recommended media, type and concentration mg l^{-1}. MS	WPM	B5	LS
Macro elements :				
NH_4NO_3	1650	400	-	1650
KNO_3	1900	-	2500	1900
$CaCl_2$	440	96.0	113.23	332.02
$MgSO_4.7H_2O$	370	370	121.56	180.54
KH_2PO_4	170	170	-	170
$(NH_4)_2SO_4$	-	-	134	-
$NaH_2PO_4.H_2O$	-	-	-	-
$Ca(NO_3)_2.4H_2O$	-	556	-	-
K_2SO_4	-	990	-	-
Micro elements : H_3BO_3	6.2	6.2	3.00	6.20
$MnSO_4.H_2O$	16.9	22.3	10.00	16.90
$ZnSO_4.7H_2O$	8.6	8.6	2.00	8.60
KI	0.83	-	0.75	0.83
$Na_2MoO_4.2H_2O$	0.25	0.025	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.025	0.025	0.025
$COCl_2.6H_2O$	0.025	-	0.025	0.025
$FeSO_4.7H_2O$	27.80	27.8	-	-
Na_2 EDTA ($2H_2O$)	37.30	37.3	36.70	36.70
Organic components: Myo-inositol	100.0	100.0	100.0	100.0
Nicotinic acid	0.50	0.5	1.00	-
Thiamine HCl	0.1	1.0	10.00	0.40
Pyridoxine HCl	0.5	0.5	1.00	-
Glycine	-	2.0	-	-

At the end of this experiment (after four weeks), the data were recorded on survival percentage, length of plantlets (cm), and number of leaves/plantlet. In this experiment, the three treatments were replicated three times, with each replicate consisting of 10 plantlets.

Experimental design and data analysis

The layout of all experiments was a completely randomized design, with two factors. Duncan's Multiple Range Test at ≤0.05 (Duncan, 1955) was used for means separation.

RESULTS AND DISCUSSION

Experiment 1: Effect of some sterilization treatments on contamination of explants

The data presented in Table (1) indicated that chlorox at concentrations of 40, 50 or 60% gave significantly higher mean percentages of contamination-free explants (65, 75 and 72.5%, respectively) than when it was applied at 30% (giving 47.5% of contamination-free explants).

Using chlorox alone at concentrations of 30, 40, 50, or 60% gave percentages of contamination-free explants ranging from 30% (with chlorox at 30 or 40%) to 50% (with chlorox at 50%). Regarding the effect of mercuric chloride (MC), the data in Table (1) show that MC concentrations of 0.2 or 0.4% were more effective in preventing contam-

ination (giving mean values of 87 and 92% of contamination-free explants respectively) than the lower MC concentrations (0 or 0.1%), which gave 37.5 and 42.5% of contamination-free explants.

When chlorox was combined with mercuric chloride (M.C.), the percentage of contamination-free explants varied from 20% to 100%. The lowest value (20%) was recorded with chlorox at 30% and M.C. at 0.1%, whereas the highest value (100%) was obtained with chlorox at 40% and M.C. at 0.4% or with chlorox at 50% or 60%, combined with M.C. at 0.2 or 0.4%. These combinations of chlorox and M.C. concentrations were the most effective treatments, giving 100% contamination-free explants in *Hydrangea macrophylla*.

This result may be due to the liability of plant tissue of *Hydrangea macrophylla* to excessive surface sterilization with M.C. which has a lysis effect on microbial cells, as stated by Russel and Chopra (1990). Also, Arafa (1992) and Hussein (2002) reported that surface sterilization with $HgCl_2$, followed by chlorox resulted in the highest decontamination of *Dieffenbachia exotica* cv. 'Tropica' and *Aglonema spp.*. The obtained data was in agreement with the findings of Hosni *et al.* (2000) on *Limonium sinuatum* var "Citron Mountain", and El-Sayed (2005) on some woody plants.

Experiment 2: Effect of BA, Kin, and TDZ on shooting behavior

Shoot number/explant

The results presented in Table (2) and Fig. (1) clearly indicate that supplementing the MS medium with different BA or TDZ concentrations (each at 0.5, 1.0, 2.0 or 4.0 mg/l), or with Kin at 2.0 or 4.0 mg/l significantly increased the number of shoot per explant, compared to the control. BA at the rate of 4.0 mg/l gave the highest mean number of shoots (3.60 shoots/explant). The general promotion of shooting on explants cultured on media containing BA, TDZ or kinetin is in agreement with the results reported by Atta-Alla and Staden (1997) on *Yucca aloifetia*, who found that the highest number of shoots was obtained with 4.5 µM TDZ plus 1.1 µM NAA. Also, El-Sawy and Bekheet (1999) on *Dieffenbachia picta* cv. 'Tropica' found that addition of BA at a concentration of 4.0 mg/l to the multiplication medium was more effective for increasing. the number of shoots than Kin.

Table (1): Effect of some sterilization treatments on contamination of Hydrangea macrophylla explants.

Mercuric chloride concentration (B)	Percentage of contamination-free explants				
	Chlorox percentage (A)				Mean (B)
	30%	40%	50%	60%	
0.0%	30 D	30 D	50 B-D	40 CD	37.5 B
0.1%	20 D	50 B-D	50 B-D	50 B-D	42.5 B
0.2%	70 A-C	80 AB	100 A	100 A	87 A
0.4%	70 A-C	100 A	100 A	100 A	92 A
Mean (A)	47.5 B	65 A	75 A	72.5 A	——

Means with different letters in the same column are significantly different ($P< 0.05$) using Duncan's multiple rang test in SAS.

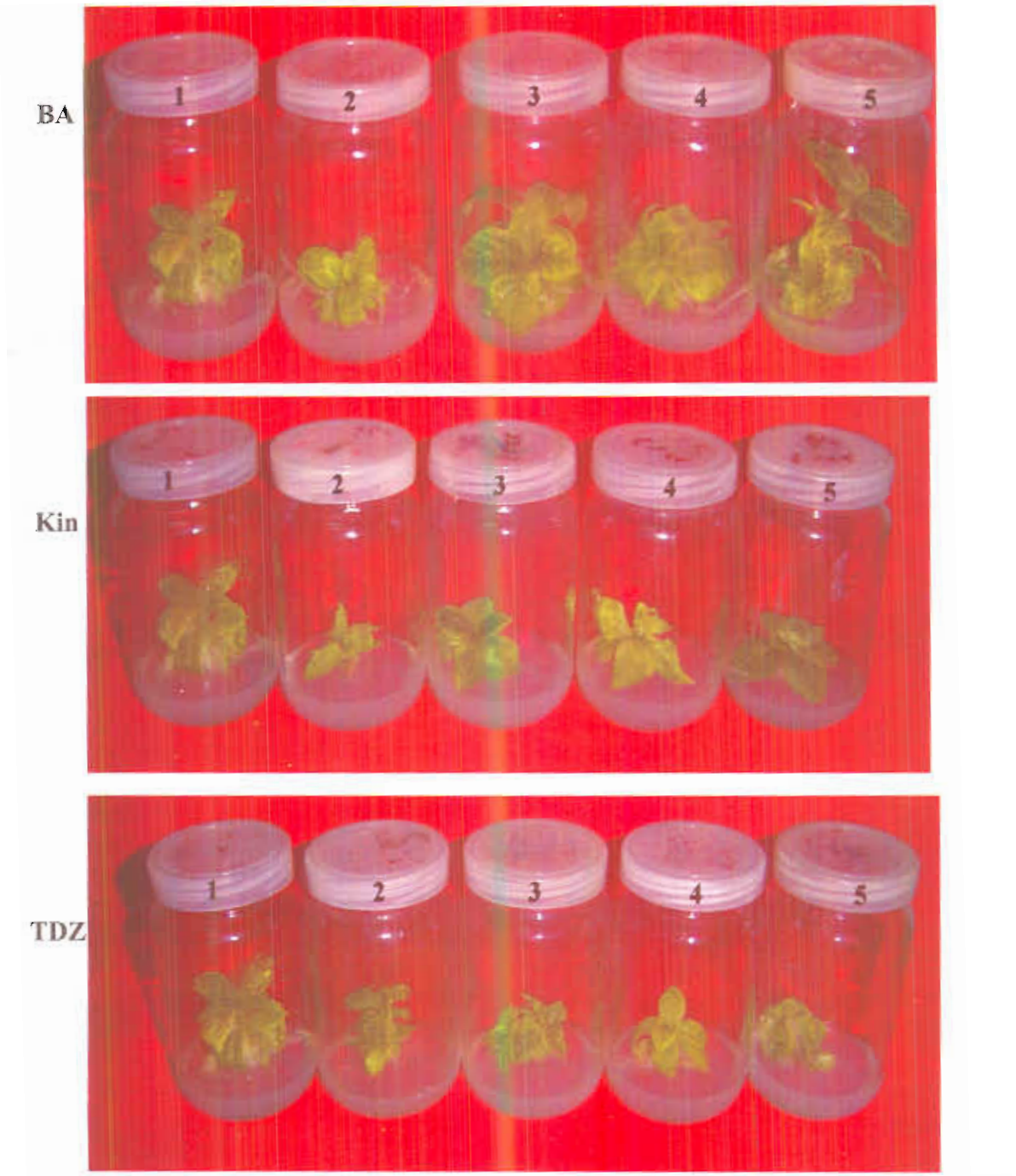

Fig. (1): Effect of BA, Kin and TDZ concentrations on shooting behavior for Hydrangea macrophylla.

1- Control (hormone free) 2- 0.5 mg/l. 3- 1.0 mg/l. 4- 2.0 mg/l. 5- 4.0 mg/l.

Table (2): Effect of BA, Kin, and TDZ concentrations on number of shoots/explant, shoot length (cm), number of leaves/explant and number of subcultures of Hydrangea macrophylla explants grown in vitro.

Culture media	Number of shoot /explants				Shoot length (cm)				Number of leaves/explant			
	No. of subcultures			Mean (A)	No. of subcultures			Mean (A)	No. of subcultures			Mean (A)
	1	2	3		1	2	3		1	2	3	
MS (control)	1.00 I	1.20 HI	1.60F-I	1.26 E	2.00 M	3.10 D-I	3.60 A-E	2.90B-D	5.60 ST	7.00 P-T	8.00 N-T	6.86 C
MS + 0.5 mg BA	1.80 F-I	2.40C-F	3.20BC	2.46 B	2.20 LM	2.90 F-K	3.60 A-E	2.90B-C	8.00 N-T	13.40 G-K	19.20 CD	13.53 C
MS + 1.0 mg BA	1.40G-I	2.80B-E	3.40 B	2.53 B	2.50I-M	3.20C-H	3.70A-D	3.13A-C	10.00 K-Q	15.00 E-I	24.80 AB	16.60B
MS + 2.0 mg BA	1.80 F-I	3.40 B	5.40 A	3.53 A	2.50 I-M	3.10 D-I	4.00 A	3.20 AB	10.80 J-P	19.60 CD	26.80 A	19.07 A
MS + 4.0 mg BA	2.40C-F	3.60 B	4.80 A	3.60 A	2.00 M	2.60 H-M	3.80 A-C	2.80C-E	13.20 H-K	22.80 BC	25.00 AB	20.33 A
MS + 0.5 mg Kin	1.20 HI	1.40 G-I	1.60F-I	1.40DE	2.40 J-M	3.00 E-J	3.30 B-G	2.90B-D	4.80 T	6.800 G-T	8.00 N-T	6.533 G
MS + 1.0 mg Kin	1.00 I	1.20 HI	1.60F-I	1.267 E	2.30K-M	3.00 E-J	3.60 A-E	2.96A-D	6.40 G-T	9.60 K-R	14.0 F-J	10.00EF
MS + 2.0 mg Kin	1.40G-I	2.40C-F	3.00B-D	2.267BC	2.70G-L	3.40 A-F	3.90 AB	3.33 A	8.40 M-T	12.49 H-L	17.20 D-G	12.67C-S
MS + 4.0 mg Kin	1.40G-I	1.80F-I	3.00B-D	2.067BC	2.50 I-M	2.90 F-K	3.50 A-F	2.96A-D	7.60 O-T	11.20 I-O	13.20 H-K	10.67D-F
MS + 0.5 mg TDZ	1.40G-I	1.80F-I	3.00B-D	2.067BC	2.30K-M	3.20 C-H	3.40 A-F	2.96A-D	6.80 G-T	11.60 I-N	18.00DE	12.13C-E
MS + 1.0 mg TDZ	1.20 HI	1.80 F-I	2.40C-F	1.80 CD	2.20 LM	2.60H-M	3.20 C-H	2.66 DE	6.00 R-T	9.20 L-S	13.20 H-K	9.467F
MS + 2.0 mg TDZ	1.40G-I	2.40C-F	3.00B-D	2.26 BC	2.10J-M	2.70 G-L	2.70 G-L	2.50 E	6.40 G-T	10.80 J-P	12.00 I-M	9.73 F
MS + 4.0 mg TDZ	2.00E-I	2.20D-G	3.00B-D	2.40 B	2.10 LM	2.60 H-M	2.60 H-M	2.43 E	8.80 L-S	16.00 D-H	17.60 D-F	14.13C
Mean (B)	1.49 C	2.18 B	3.00 A	—	2.29 C	2.94 B	3.45 A	—	7.90 C	12.72 B	16.69 A	—

Within the same column, means followed with different letters are significantly different ($P< 0.05$), according to Duncan's multiple range test.

Moreover, El-Sawy *et al.* (2000) on *Dracena masengana* cv. 'Tricolour' obtained the highest number of shoots/explant on an MS medium supplemented with 4.0 mg/l of BA. Abou Dahab *et al.* (2004) found that *Aspidistra elatior* cultured on an MS medium supplemented with TDZ at 4.5 mg/l produced the highest number of shoots/explant. Preece and Ledbetter (2003) studied the influence of thidizuron (TDZ) on *in vitro* shoot proliferation of Oakleaf hydrangea; they found that the total shoot production was greatest at 10^{-6} TDZ, with a mean of 5.2 shoots at this concentration. There was a combination of axillary and adventitious shoots, but as the concentration of TDZ decreased there were predominately axillary shoots.

The data in Table (2) also show that increasing the number of subcultures significantly increased shoot formation on the explants.

Regarding the interaction between the effects of BA, Kin, and TDZ and that of the number of subcultures, the recorded data showed that in the first and second subcultures, the highest number of shoots (2.40 and 3.60 shoots/explants, in the first and second subcultures, respectively) was recorded on the MS medium supplemented with BA at the rate of 4.0 mg/l, but in the third subculture, BA at the rate of 2.0 mg/l gave the highest value (5.40 shoots/explant), compared to the control and to the other treatments. Using an MS medium free of hormones (control) gave the lowest number of shoots (1.0, 1.2 and 1.6 shoots/explant in the three subcultures, respectively).

Shoot length

The results recorded on *Hydrangea macrophylla* explants (Table 2) show that using TDZ at the concentrations of 2.0 and 4.0 mg/l significantly reduced shoot length, compared to the control. In contrast, the longest shoots (3.33 cm) were recorded on MS medium plus 2.0 mg/l Kin. A significant increase in shoot length was recorded with increasing the number of subcultures. Regarding the interaction between the effects of growth regulators (BA, Kin and TDZ) and the number of subcultures, the recorded data indicated that in the first and second subcultures, the longest shoots (2.70 and 3.40 cm, respectively) were recorded on MS medium supplemented with 2.0 mg/l Kin. In the third subculture, the MS medium supplemented with 2.0 mg/l BA resulted in the longest shoots (4.0 cm), whereas MS medium supplemented with 2.0 mg/l TDZ gave the shortest shoots (2.70 cm). The reduction in shoot length due to TDZ treatments at the rate of 1.0, 2.0, or 4.0 mg/l was also recorded by Preece and Ledbetter (2003) on *Hydrangea quercifolia.*

In conclusion, it can be stated that BA at the rate of 2.0 mg/l or Kin at the rate of 2.0 mg/l were the most effective treatments in producing the longest shoots.

Number of leaves/explants.

It is clear from the data in Table (2) that the highest number of leaves (20.33 leaves/explants) was recorded on explants cultured on MS medium supplemented with BA at the rate of 4.0 mg/l, followed by MS medium supplemented with BA at 2.0 mg/l (giving 19.07 leaves/explant), with no significant difference between these two treatments. On the other hand, the lowest number of leaves (6.86 leaves/explant) was recorded on control explants (grown on MS medium free of hormones). Also, the results indicated that as the number of subcultures increased, a significant increase in the number of leaves per explants was obtained.

Data on the interaction between the growth regulator treatments and the number of subcultures indicated that the highest number

of leaves was recorded when the MS medium was supplemented with BA at the rates of 2.0 mg/l (in the third subculture) or 4.0 mg/l (in the first and second subcultures).

The above results are in agreement with the findings of Abou Dahab *et al.* (2004) on *Aspidistra elatior*. They observed that the highest number of leaves was recorded by culturing the explants on MS medium plus 6.0 mg/l BA and 1.0 mg/l Kin. Using an MS medium free of hormones (control) significantly decreased the number of leaves.

Experiment 3: Effect of different media on shooting behavior

Number of shoots/explant.

The data in Table (3) and Fig. (2) indicate that the different media used in this study had a significant effect on number of shoots/explant. The mean number of shoots formed on *Hydrangea macrophylla* explants varied from 3.46 to 5.26 shoots/explant, depending on the medium used. The highest number of shoots was formed on explants grown on the full-strength B5 medium, followed by explants grown on the half-strength LS medium. On the other hand, the lowest number of shoots was produced by explants grown on the half-strength WPM medium.

In general, one can say that using the full-strength B5 medium or the half-strength LS medium were the most effective treatments in promoting shoot initiation and development, compared to the use of MS and WPM media. One can also observe that no significant difference was recorded between the numbers of shoots formed on explants cultured on MS or WPM media, regardless of their concentration (full- or half-strength).

Regarding the effect of number of subcultures on shoot formation by *Hydrangea macrophylla* explants, the data in Table (3) show that increasing the number of subcultures significantly increased the number of shoots/explant.

The above conclusions are in agreement with the findings of Douglas *et al.* (1986) on *Hydrangea macrophylla in vitro* propagation. Regarding the interaction between the effects of culture media and number of subcultures, the data in Table (3) show that the full-strength B5 medium gave the highest number of shoots after the 3rd subculture (9.60 shoots/explant). It can therefore be recommended that, for production of the highest number of shoots, *Hydrangea macrophylla* explants should be cultured on a full-strength B5 medium, and subcultured 3 times.

Shoot length

Significant differences were detected between means of the tested media in their effects on shoot length. The longest shoots (3.86 cm) were found when using the half-strength B5 medium, followed by the half-strength LS medium (3.50 cm), whereas the shortest shoots (2.96 cm) were produced when the full-strength WPM medium was used.

The data in Table (3) also revealed significant increases in shoot length as number subcultures was increased. The longest shoots (3.93 cm) were obtained after the 3rd subculture. Data recorded after each subculture on explants grown on the different media have shown that in the 1st subculture, no significant differences were observed between full and half strengths of the different media. It was also observed that the longest shoots (4.70 cm) were obtained after the third subculture on explants cultured on the half-strength B5 medium. Thus, it can be concluded that the best treatment for increasing the shoot length of *Hydrangea macrophylla* was using a half-strength B5 medium. Similar conclusions were reached by Gabr (2004) on *Ruscus hypoglossum*, and Douglas *et al.* (1986) on *Hydrangea macrophylla*.

Number of leaves/explant

The highest number of leaves (29.60 leaves/explant) was recorded by culturing the explants on full-strength B5 medium, whereas the lowest number of leaves (18.67 leaves/explant) was recorded on the half-strength MS medium.

There was a significant increase in the number of leaves/explant as the number of subcultures increased. The data indicated that after the 1st subculture, explants grown on half-strength B5 medium gave the highest number of leaves (11.6 leaves/explant), whereas the lowest number of leaves (8.40 leaves/explant) was obtained on explants grown on the half-strength MS medium. However, in the 2nd and 3rd subcultures, the greatest number of leaves (33.60 and 45.20 leaves/explant) resulted from using the full-strength B5 medium. Thus, it can be concluded that full strength B5 medium was the best treatment for producing the greatest number of leaves/explants.

Experiment (4): Effect of IBA and activated charcoal on rooting behavior and vegetative development

Number of roots/explant

The results shown in Table (4) and Fig. (3) revealed that the mean number of roots varied from 4.0 to 13.75 roots/plantlet, depending on the IBA concentration. The number of roots increased significantly by increasing the concentrations of IBA from 0.0 to 2.0 mg/l. Thus, the lowest mean value (4.00 roots/plantlet) was found for explants grown on the half-strength MS medium containing no IBA (control), whereas supplementing the medium with IBA at 2.0 mg/l gave significantly more roots (13.75 roots/plantlet) than any other treatment, including the control. The percentage of increase in number of roots caused by using IBA at 2.0 mg/l (compared to the control) was (243.75%). Raising IBA concentration from 2.0 to 3.0 mg/l caused a significant reduction in number of roots/plantlet.

Concerning the effect of activated charcoal (A.C.), the data in Table (4) show that using activated charcoal gave significantly more roots (10.50 roots/plantlet), than those obtained without charcoal (7.75 roots/plantlet). This represents an increase of 35.48% in the number of roots formed as a result of using A.C., compared to that obtained without A.C.

Concerning the interaction between the effects of IBA and activated charcoal, the recorded data showed that the highest number of roots (16.00 roots/plantlet) was recorded when the explants were cultured on a half-strength MS medium supplemented with 2.0 mg/l IBA in the presence of activated charcoal, while the lowest number of roots (3.50 roots/plantlet) was recorded when growing on a medium without IBA, and without activated charcoal (control).

Tabel (3): Effect of different media (MS, WPM, B5, LS) on shooting behavior of Hydrangea macrophylla grown in vitro.

Culturing media (A)	Number of shoots/explant				Shoot length (cm)				Number of leaves/explant			
	No. of subcultures (B)			Mean (A)	No. of subcultures (B)			Mean (A)	No. of subcultures (B)			Mean (A)
	1	2	3		1	2	3		1	2	3	
Full MS	2.40 J-L	3.60 G-J	4.80 D-G	**3.60 C**	2.40 LM	3.10 G-J	3.800 C-E	**3.10 C**	8.80 I	18.40 H	25.60 BC	**20.93 B-D**
Half MS	2.60 I-L	3.40 H-K	5.20 D-F	**3.73 C**	2.80 I-L	3.40 E-H	3.60 D-G	**3.26 BC**	8.40 I	20.80 GH	26.80 D-F	**18.67 D**
Full WPM	1.80 L	3.60 G-J	5.40 C-E	**3.60 C**	2.20 M	3.20 F-I	3.50 E-H	**2.96 C**	10.00 I	28.00 DE	31.20 CD	**23.07 BC**
Half WPM	2.40 J-L	3.20 H-K	4.80 D-G	**3.46 C**	2.60 J-M	3.00 H-K	3.50 E-H	**3.03 C**	10.80 I	22.40 F-H	36.80 B	**23.33 B**
Full B5	2.20 KL	4.00 F-H	9.60 A	**5.26 A**	2.60 J-M	3.40 E-H	3.70 C-F	**3.23 BC**	10.00 I	33.60 BC	45.20 A	**29.60 A**
Half B5	2.20 KL	3.80 G-I	5.80 B-D	**3.93 BC**	2.80 I-L	4.10 B-D	4.70 A	**3.86 A**	11.60 I	24.40 E-G	34.00 BC	**23.33 B**
Full LS	2.20 KL	3.40 H-K	6.60 BC	**4.00 BC**	2.50 K-M	3.60 D-G	4.20 A-C	**3.43 B**	9.20 I	22.40 F-H	37.60 B	**23.07 BC**
Half LS	2.60 I-L	4.20 E-H	7.00 B	**4.60 AB**	2.60 J-M	3.40 E-H	4.50 AB	**3.50 B**	8.80 I	20.40 GH	31.20 CD	**20.13 CD**
Mean (B)	**2.30 C**	**3.65 B**	**6.15 A**	___	**2.56 C**	**3.40 B**	**3.93 A**	___	**9.70 C**	**23.80 B**	**34.80 A**	___

Within the same column, means followed with diffcrent letters are significantly different (P< 0.05), according to Duncan's multiple range test.

Fig. (2): Effect of media type salt strength on shooting behavior for Hydrangea macrophylla.

1. Full B5 salt strength. 2. Half B5 salt strength.

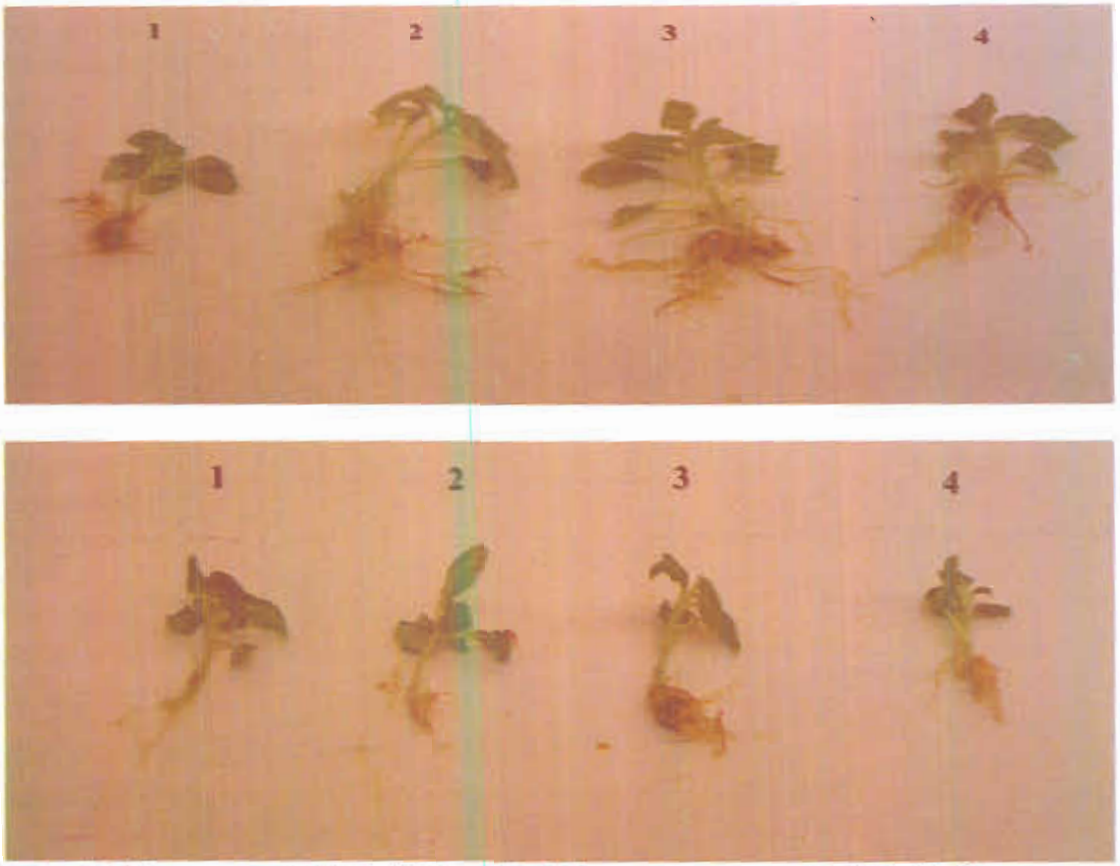

Fig. (3): Effect of IBA concentrations with and without activated charcoal (A.C.) on root formation and shootlet growth of Hydrangea macrophylla.

1: 1/2 MS free IBA (control). 2: 1/2 MS + 1.0 mg/l IBA. 3: 1/2 MS + 2.0 mg/l IBA. 4: 1/2 MS + 3.0 mg/l IBA.

Similar conclusions were reached by Gabr (2004) on *Ruscus hypoglossum*, and Douglas *et al.* (1986) on *Hydrangea macrophylla*.

Number of leaves/explant

The highest number of leaves (29.60 leaves/explant) was recorded by culturing the explants on full-strength B5 medium, whereas the lowest number of leaves (18.67 leaves/explant) was recorded on the half-strength MS medium.

There was a significant increase in the number of leaves/explant as the number of subcultures increased. The data indicated that after the 1st subculture, explants grown on half-strength B5 medium gave the highest number of leaves (11.6 leaves/explant), whereas the lowest number of leaves (8.40 leaves/explant) was obtained on explants grown on the half-strength MS medium. However, in the 2nd and 3rd subcultures, the greatest number of leaves (33.60 and 45.20 leaves/explant) resulted from using the full-strength B5 medium. Thus, it can be concluded that full strength B5 medium was the best treatment for producing the greatest number of leaves/explants.

Experiment (4): Effect of IBA and activated charcoal on rooting behavior and vegetative development

Number of roots/explant

The results shown in Table (4) and Fig. (3) revealed that the mean number of roots varied from 4.0 to 13.75 roots/plantlet, depending on the IBA concentration. The number of roots increased significantly by increasing the concentrations of IBA from 0.0 to 2.0 mg/l. Thus, the lowest mean value (4.00 roots/plantlet) was found for explants grown on the half-strength MS medium containing no IBA (control), whereas supplementing the medium with IBA at 2.0 mg/l gave significantly more roots (13.75 roots/plantlet) than any other treatment, including the control. The percentage of increase in number of roots caused by using IBA at 2.0 mg/l (compared to the control) was (243.75%). Raising IBA concentration from 2.0 to 3.0 mg/l caused a significant reduction in number of roots/plantlet.

Concerning the effect of activated charcoal (A.C.), the data in Table (4) show that using activated charcoal gave significantly more roots (10.50 roots/plantlet), than those obtained without charcoal (7.75 roots/plantlet). This represents an increase of 35.48% in the number of roots formed as a result of using A.C., compared to that obtained without A.C.

Concerning the interaction between the effects of IBA and activated charcoal, the recorded data showed that the highest number of roots (16.00 roots/plantlet) was recorded when the explants were cultured on a half-strength MS medium supplemented with 2.0 mg/l IBA in the presence of activated charcoal, while the lowest number of roots (3.50 roots/plantlet) was recorded when growing on a medium without IBA, and without activated charcoal (control).

In conclusion, it can be recommended that for obtaining the highest number of roots/plantlet, *Hydrangea macrophylla* explants should be cultured on a half-strength MS medium, supplemented with 2.0 mg/l IBA and containing activated charcoal. In this regard, Chen and Bit Hua (2000) showed that rooting of *Spathiphyllum xiangshui* was best on a ½ MS medium containing 0.5 mg/l IBA + 0.5 g/l activated charcoal.

2- Root length (cm)

The data in Table (4) showed that the longest roots (7.00 cm) were obtained when the half-strength MS medium was supplemented with 2.0 mg IBA/l.

Concerning the effect of activated charcoal, it is clear that the roots were significantly longer (5.56 cm) when activated charcoal was used, compared to root length obtained on a medium containing no activated charcoal (4.25 cm).The data recorded on the interaction between the effects of IBA and activated charcoal revealed that the longest roots (7.50 cm) were recorded when culturing the explants on the half-strength MS medium supplemented with 2.0 mg/l IBA in the presence of activated charcoal, while the shortest roots (2.00 cm) were recorded when the explants were cultured on a medium containing no IBA or activated charcoal (control). In conclusion, it can be stated that the tallest roots were recorded when the half-strength MS medium was supplemented with 2.0 mg/l IBA in the presence of activated charcoal. In this regard, Du-Xue Mei *et al.* (1997) on *Jujube ohtained* the best results on media containing 20 mg/l IBA.

Plantlet height

The recorded data indicated that the tallest plantlets (4.75 cm) were obtained when the half-strength MS medium was supplemented with 2.0 mg/l IBA. Also, the results presented in Table (4) also show that addition of activated charcoal to the medium gave significantly taller plantlets (4.31 cm tall) than those grown without activated charcoal (with a mean height of 3.43 cm).

Among the different combinations of IBA and activated charcoal treatments, combining IBA at 3.0 mg/l with the addition of activated charcoal gave the tallest plants (5.25 cm). Along the same lines, OrlioKwska *et al.* (2000) reported that during *in vitro* rooting of *Codiaeum variegatum* cv. 'Excellent', decreasing the IBA concentration to 0.5 mg/l to stimulated shoot elongation.

Number of leaves/plantlet

The data recorded on *Hydrangea macrophylla* plantlets (Table 4) indicated that the highest mean number of leaves (12.75 leaves/plantlet) was obtained on a half-strength MS medium containing 2.0 mg IBA/l, followed by a medium supplemented with 3.0 mg IBA/l (giving 12.25 leaves/plantlet), with no significant difference between the two treatments. On the other hand, the lowest number of leaves (6.50 leaves/plantlet) was produced by using a medium containing no IBA.

Moreover, adding activated charcoal to the half-strength MS medium significantly increased the number of leaves/plant (giving 11.13 leaves/plantlet), as compared with a medium free of charcoal (giving 8.87 leaves/plantlet). This trend is similar to that observed for the number of roots/plantlet, root length and plantlet height.

Among the different combinations of IBA and activated charcoal treatments, the highest number of leaves (14.0 leaves/plantlet) was obtained when the explants were cultured on an MS medium supplemented with 2.0 mg IBA/l, in the presence of charcoal.

Experiment (5): Effect of some growing media on acclimatization of *Hydrangea macrophylla*

Survival percentage (Table 5)

The recorded results (Table 5) show that the different growing media used in this study had a significant effect on the survival percentage. When the acclimatization medium consisted of peat moss or peat moss + sand (1:1 v/v), the survival percentage was 100%. On the other hand, the lowest value (33%) was recorded with peat moss + perlite (1:1 v/v).

Plantlet height

It is evident from the recorded data (Table 5) that there was no significant difference

Table (4): Effect of IBA concentrations and activated charcoal on rooting and shootlet growth of Hydrangea macrophylla grown in vitro.

Culturing media	Number of roots/plantlet		Mean (A)	Root length (cm)		Mean (A)	Plantlet height (cm)		Mean (A)	No. of leaves/plantlet		Mean (A)
	With Activated charcoal	Without Activated charcoal		With Activated charcoal	Without Activated charcoal		With Activated charcoal	Without Activated charcoal		With Activated charcoal	Without Activated charcoal	
½ MS (control)	4.50 EF	3.50 F	4.00 D	3.25 C	2.00 F	2.62 D	3.25 F	2.25 G	2.75 D	7.50 D	5.50 E	6.50 C
½ MS + 1.0 mg/l IBA	7.00 D	5.50 E	6.25 C	4.50 D	2.75 E	3.62 C	3.75 E	3.25 F	3.50 C	9.50 C	7.50 D	8.50 B
½ MS+ 2.0 mg/l IBA	16.00 A	11.50 C	13.75A	7.50 A	6.50 B	7.00 A	5.25 A	4.25 C	4.75 A	14.00 A	11.50 B	12.75A
½ MS+ 3.0 mg/l IBA	14.50 B	10.50 C	12.50B	7.00 AB	5.75 C	6.37 B	5.00 B	4.00 D	4.50 B	13.50 A	11.00 B	12.25A
Mean (B)	10.50 A	7.75 B	____	5.56 A	4.25 B	____	4.31 A	3.43 B	____	11.13 A	8.87 B	____

Within the same column, means followed with different letters are significantly different ($P< 0.05$), according to Duncan's multiple range test.

the height of plantlets acclimatized in peat moss, peat moss + sand (1:1 v/v), or peat moss + vermiculite (1:1 v/v). The tallest plantlets (11.33 cm) were those acclimatized in peat moss + sand (1:1 v/v). On the other hand, plantlets acclimatized in peat moss + perlite (1:1 v/v) were significantly shorter than those acclimatized in any other medium.

The data in Table (5) show that there was no significant difference between the heights of plantlets acclimatized in peat moss, peat moss + sand, or peat moss + vermiculite (giving plantlet heights of 12.00, 12.67 and 10.67 cm, respectively). However, plantlets grown in any of these media were significantly taller than those acclimatized in peat moss + perlite (with a height of 3.33 cm). on decreased the number of leaves/plant.

Number of leaves/plantlet

Table (5): Effect of growing media during adaptation stage on Hydrangea macrophylla.

Treatments	Survival %	Plantlet length (cm)	Number of leaves/plantlet
Peat moss	100 A	9.66 A	12.00 A
Peat moss + sand (1:1)	100 A	11.33 A	12.67 A
Peat moss + vermiculite (1:1)	60 B	8.66 A	10.67 A
Peat moss + perlite (1:1)	33 C	3.00 B	3.33 B

Within the same column, means with different letters are significantly different (P< 0.05), according to Duncan's multiple range test.

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الملخص العربي

إكثار نباتات الهيدرانجيا بطريقة زراعة الأنسجة

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أجرى فى هذا البحث خمسة تجارب معملية باستخدام فصلات نباتية من نباتات الهيدرانجيا وذلك لغرض عمل بروتوكول للإكثار الدقيق لنباتات الهيدرانجيا. التجربة الأولى: وجد أن استخدام التعقيم بالكلوركس بتركيز ٥٠% مضاف إليه كلوريد الزئبقيق (MC) بتركيز ٠,٢% أعطى أعلى نسبة من الفصلات النباتية الخالية من التلوث (١٠٠%).
و أعطت نتائج التجربة الثانية: تأثير بعض السيتوكينينات (BA, Kin, TDZ) بتركيزات مختلفة على سلوك الإكثار الدقيق و تكوين السيقان. و أظهرت النتائج إن استخدام بيئة MS مضاف إليها BA بتركيز ٢ مجم/لتر أعطت أعلى النتائج بالنسبة لعدد السيقان النامية. كما إن زيادة عدد مرات النقلات أدى إلى زيادة معنوية فى هذه الصفة. و إن النقلة الثالثة أعطت أفضل النتائج. أدى إضافة TDZ إلى بيئة MS إلى نقص معنوى فى طول السيقان النامية و عموما فان بيئة MS المضاف إليها BA أو Kin بتركيز ٢ مجم/لتر كانت أفضل المعاملات حيث أعطت زيادة معنوية فى طول السيقان النامية. كما إن زراعة الفصلات النباتية على بيئة MS مضافا إليها BA بتركيز ٤مجم/لتر أعطت أفضل النتائج بالنسبة لعدد الأوراق على الفصلة النباتية.
أظهرت نتائج التجربة الثالثة: إن استخدام التركيز الكامل من بيئة B5 كان أفضل البيئات معنويا من حيث عدد السيقان, زيادة عدد النقلات أدى إلى زيادة معنوية فى هذه الصفة, كما إن أطول السيقان نتجت عند النقلة الثالثة مع استعمال التركيز الكامل من بيئة B5. يعتبر التركيز نصف الكامل من بيئة B5 أفضل البيئات بالنسبة لطول السيقان فى الثلاث نقلات, كما أعطى استخدام التركيز الكامل من بيئة B5 أفضل النتائج بالنسبة لعدد الأوراق. و بينت نتائج التجربة الرابعة: تأثير استخدام IBA و الفحم النشط على تكوين الجذور و قد أعطى اكبر عدد من الجذور المتكونة و كذلك أطول الجذور و أمكن الحصول عليه عند استخدام بيئة MS بنصف تركيز مضاف إليها IBA بتركيز ٢ مجم/لتر مع الفحم النشط, بينما كان أطول النبتات الناتجة عند استخدام بيئة MS مضاف إليها IBA بتركيز ٢ أو ٣ مجم/لتر.
و أظهرت نتائج التجربة الخامسة: خلال فترة الأقلمة إن أطول النباتات و أكثر عدد من الأوراق أمكن الحصول عليه عند استخدام بيئة مكونة من البيت موس مع الرمل بنسبة (١:١بالحجم) كما إن نسبة نجاح النبتات وصلت إلى ١٠٠%.