

## SELECTION OF SALT TOLERANT CLONES OF SOME MEDICINAL PLANTS THROUGH TISSUE CULTURE TECHNIQUE:

### I. *Hyoscyamus muticus* L. PLANT.

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### ABSTRACT

Response of Egyptian henbane plant callus (*Hyoscyamus muticus* L.) to various levels of salt was investigated. Two tolerant clones to salt were selected from callus cultured on selection medium contained 6000 and 8000 ppm of salts, respectively. Tolerant clones were capable of proliferating and growing on media contained the same salt concentrations and supplemented with 1.5 ppm of BA. Vegetative characters were significantly enhanced in general at the lower salt concentration (6000 ppm) especially with the media containing 0.12 g/ L  $\text{KH}_2\text{PO}_4$  +4 ppm kinetin + 0.5 ppm IBA. Using of MS media at its half strength with 1ppm NAA was the best for henbane root growth at 6000ppm of salt. Survival percentage reached up to 28.00 % for clone A(selected at 6000ppm ) and 32.02 % for clone B(selected at 8000ppm) with a total alkaloid percentage of 0.90 and 0.98 %, respectively .

**Keywords:** Salt tolerant clones - Tissue culture technique - *Hyoscyamus muticus*

### INTRODUCTION

One of the most significant trends in plant biotechnology is to produce cultivars tolerant to unfavorable environmental conditions including salt stress.

However, only little success is attained using Conventional breeding methods for enhancing the response of plants to saline tension. Tissue culture techniques have widely been employed for production of plants bearing specific traits including disease resistance, cold tolerance and salt tolerance. The main idea of using tissue culture in a biotic experiments is based on the fact that cell is used as a selection unit instead of the whole plant. Various plant species have successfully been manipulated in vitro under salt strain conditions and finally salt - tolerant plants were recovered (Garcia – Reina, *et al* 1988 and Barakat and Abdelatif 1995).

Henbane is one of the most common plants in the family Solanaceae (Dhoot and Hpnshaw, 1977). The plant occurs in the desert in patches occupying depressions in sandy areas which receive runoff water. It grows in areas where the measured rainfall averages 20 mm or more per year. Water runoff increases the water revenue in habitats supporting the wild plant. However, the supply from wild plants is not sufficient for industrialization.

Due to the lack of available knowledge study the production of salt - tolerant clone of henbane, this present work focuses on applying selection of salt - resistant cells of *Hyoscyamus muticus* callus cultures and forcing this selected callus to proliferate into shoots leaves and roots under the same levels of salts. The present work is based on many previous researchers

whom have been working in this field of study using different techniques as Davis *et al* (1977) with his work on carnation, pointed out the role of using NAA at 1 ppm and kinetin at 10 ppm to improve the growth of carnation shoot tips. El-Tarras (1995) with Banana, declared the importance of using electrophoresis technique to diminish the possibility of deformed banana plantlets production. Maged (1996) with *Cucumis melo*, succeeded to produce a colne tolerant to sodium chloride from callus of Cantaloupe. Zhao *et al* (2001), mentioned the role of addition of KCl, mannitol, and synthetic precursors and bioregulators on improving indole alkaloid production of *Catharanthus roseus* callus culture. Ouf *et al* (2003) with *Sorghum bicolor*, utilized the somaclonal variation tool to produce new high productive cultivars of sorghum. Al-Wasel (2006), studied the role of using growth regulators (BA, GA3, IBA, and IAA) on improving nodal segments growth, proliferation and rooting of *Atropa belladonna* plant.

It is hoped that this study will provide the necessary information for selection salt tolerant clones of henbane and for further investigations on understanding biological basis of salt resistance in plants.

## **MATERIALS AND METHODS**

### **1- Plant material**

Callus was initiated from mature seeds of *Hyoscyamus muticus* L obtained from the Experimental farm of Faculty of Pharmacy, Cairo University. The seeds were surface sterilized in commercial hypochlorite solution (Clorox) at concentration of 30 % for 20% minutes, then washed three times with sterilized distilled water. Sterilized seeds were cultured onto surface of Murashige and Skoog media (1962). Twenty seeds had been cultured in each experimental 250 ml jar. The medium was supplemented with Glycine(1mg / L), Thiamine (1mg / L), Nicotinic acid (1mg / L), pyridoxine (1mg / L), and Myo-Inositol (1mg / L). Growth regulators were added to the culture medium depending on the stage of culture as following:

#### **a) Callus initiation**

The media used for henbane callus initiation is based mainly on using MS medium supplemented with 2.5 ppm GA+ 2ppm CaPantothenic acid for all treatments except with control treatment.

The previous media composition was amended with the desired growth regulators under investigation as following :

BA at 0.5, 1 and 1.5 ppm.

2,4-D at 1 and 5 ppm .

NAA at 1 and 5 ppm.

B ) Callus Differentiation and proliferation

The medium used for callus proliferation was mainly MS media with 0.12g/L KH<sub>2</sub>PO<sub>4</sub> plus the growth regulators under investigations as following: Kinetin at 4ppm in combination with IBA at 0.5 ppm or NAA at 0.5 ppm.

#### **C) Root growth**

Root growth has been studied using different types of media as following:

- MS medium at full strength (control).
- MS medium at half strength.
- MS medium at half strength supplemented with NAA at 1 ppm or IBA at 1ppm.

Following adjustment of the pH at (5.8), 3 % sucrose and 7 g / L agar were added to the culture medium. The media were autoclaved for 20 min at 1.5 bars then kept in light at room temperature until use.

The whole work has been done at the experimental laboratory of tissue culture, Horticulture Department, Faculty of Agriculture, Ain Shams University, Shoubra El –Keima, Cairo, Egypt.

#### **D) Acclimatization**

Successful plantlets had been adapted to acclimatization stage using direct exposure of cultivated jars to normal environmental conditions for one week. The plantlets brought out the jars and washed to free them from medium's agar. Finally, the plantlets cultured in foam pots contain peat: sand at ratio of 1 :1.

#### **2) Salt Selection procedures**

Inhibitory concentrations were determined by transferring small pieces of callus on surface of nutrient medium containing Sodium chloride, Magnesium chloride and Potassium chloride at the ratio of [1 NaCl : 3 ( 1 MgCl: 3 KCl)] and the salt concentrations of: 0, 2000, 4000, 6000, 8000 up to 16000ppm.

The cultures were incubated at  $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$  for 45 days. Callus fresh weight was determined according to Meredith (1978).

Having determined the lowest inhibitory concentrations selection was carried out using concentrations higher than that known to have inhibitory effect.

#### **3) Alkaloids content determination**

The total percentage of alkaloids content was determined using plantlets fresh leaves based on Atropine alkaloid determination method mentioned by Foster and Cornelia (1970).

#### **4) Statistical analysis**

The experiment has been designed using a complete randomized design with four replicates of each treatment and each replicate had four jars. Data were statistically analyzed according to Snedecor and Cochran (1967). Comparisons among means were made via the least significant difference multiple ranges (LSD) and Duncan student test.

## **RESULTS AND DISCUSSION**

#### **- Growth of callus**

Callus initiation of henbane seeds was significantly enhanced by using BA compared with the other growth regulators have been investigated. 2,4-D and NAA had an effect on callus growth compared to the control treatment but without significance compared with the BA treatments as shown in Table(1).

It is evident that using MS media supplemented with BA at 1 ppm in combination with GA at 2.5 ppm and CaPantothenic acid at 2 ppm gave a

significant value of callus initiation (11.06 clone/ jar). The other BA concentrations (0.5 and 1.5 ppm), 2,4-D and NAA at various concentrations did not show a significant difference compared with the control.

These findings agree with those of Ghanem (1994) who stated that growth regulators plays an important role on the morphogenesis response in vitro of henbane plant due to their influence on cell division, cell elongation and enlargement.

Similar results had been mentioned also by Al-Wasel (2006), who used Murashige and Skoog media supplemented with BA at (0.5 - 2.5 ppm) to induce multiple shoot formation of *Atropa belladonna* L. plant

**Table (1): Callus initiation four weeks after sowing under different MS media regimes supplemented with different growth regulators of *Hyoscyamus muticus* seeds**

Media type	Callus initiation ( No of clones per jar)
Control ( MS media free of growth regulators)(	0,00 <sup>d</sup>
MS+ 2.5 ppm GA+2 ppm CaPantothenic acid+ 0.5 ppm BA	7,37 <sup>b</sup>
MS+ 2.5 ppm GA+2 ppm CaPantothenic acid+ 1 ppm BA	11,06 <sup>a</sup>
MS+ 2.5 ppm GA+2 ppm CaPantothenic acid+1.5 ppm BA	9,43 <sup>ab</sup>
MS+ 2.5 ppm GA+2 ppm CaPantothenic acid+ 1 ppm 2,4D	3,00 <sup>c</sup>
MS+ 2.5 ppm GA+2 ppm CaPantothenic acid+5 ppm 2,4D	4,37 <sup>c</sup>
MS+ 2.5 ppm GA+2 ppm CaPantothenic acid+1 ppm NAA	3,93 <sup>c</sup>
MS+ 2.5 ppm GA+2 ppm CaPantothenic acid+ 5 ppm NAA	3,20 <sup>c</sup>

Table (2) indicates the influence of direct exposure of callus portions to salt concentrations. Data clarifies the role of using the growth regulators to improve henbane cells tolerance to salts. Callus growth was significantly enhanced at the lower concentration of salt (4000 ppm) compared with the higher ones (6000 and 8000 ppm). Using of BA at 0.5 ppm has a significant effect on callus growth reached up to 1.58 clones/ jar. However, the interaction of using BA at 0.5 ppm with salts has the same trend of giving high significant values of callus growth at the lowest level of salt (4000 ppm).

**Table (2): *Hyoscyamus muticus* callus growth under different salt concentrations and growth regulator treatments.**

Salt concentrations.	4000 ppm	6000 ppm	8000 ppm	Mean
	Average number of clones/ jar			
Media type.				
Control ( MS media)	0.50 <sup>c</sup>	0.50 <sup>c</sup>	0.00 <sup>c</sup>	0.16 <sup>d</sup>
MS + 2.5 GA + 2 ppm CaPantothenic acid + 0.5 ppm BA	3.25 <sup>a</sup>	1.50 <sup>b</sup>	0.00 <sup>c</sup>	1.58 <sup>a</sup>
MS + 2.5 GA + 2 ppm CaPantothenic acid + 1 ppm BA	3.25 <sup>a</sup>	0.25 <sup>c</sup>	0.00 <sup>c</sup>	1.16 <sup>b</sup>
MS + 2.5 GA + 2 ppm CaPantothenic acid + 1.5 ppm BA	1.25 <sup>b</sup>	0.50 <sup>c</sup>	0.00 <sup>c</sup>	0.66 <sup>cd</sup>
MS + 2.5 GA + 2 ppm CaPantothenic acid + 5 ppm 2,4-D	0.25 <sup>c</sup>	0.00 <sup>c</sup>	0.25 <sup>c</sup>	0.33 <sup>c</sup>
Mean	1.75 <sup>a</sup>	0.55 <sup>b</sup>	0.05 <sup>c</sup>	

Table (3) data shows the effect of gradual exposure of successfully selected henbane callus clones on 4000 ppm salt concentration. Clones selected at 4000 ppm were cultured on media containing higher salt concentrations as following 8000, 12000, and 16000 ppm.



Basically, callus clones grew well with MS media supplemented with 8000 ppm salts compared with the other higher concentrations, 12000 and 16000 ppm. Significant increases of number of callus clones per jar, callus fresh weight/g and callus dry weight/g have been achieved with the lowest salt concentration (8000 ppm) and were 2.00, 9.45 and 2.09 respectively. Also using of growth regulators had a significant effect for different callus characters especially with adding BA to the medium at 0.5 ppm plus 2.5 ppm of GA and 2 ppm of CaPantothenic acid.

Concerning the interaction between it could be observed that salinity and media composition, the same trend has been observed concerning the role of growth regulators on improving callus capability for salt tolerance. The callus fresh weight has significantly enhanced by using BA at 0.5, 1, and 1.5 ppm with values of 11.12, 10.27, and 13.27 g, respectively.

These findings may be attributed to the changes in the endogenous hormones in the cultured tissue as well as the influence of the period of culture (Murashige, 1977).

In this experiment, the same trend has been observed with proliferation of callus under different levels of salt.

The medium containing 1.5 ppm BA in combination with 2.5 ppm GA enhanced shoot proliferation compared with the other media contain Kinetin and/or IBA. The most significant effect induced by BA at the lower salt concentration (8000 ppm) as shown in Table (4).

Table (4) shows that increasing BA concentration to 1.5 ppm significantly improves henbane callus proliferation to bud primorida with value reached up to 2.75 proliferated clones/ jar. BA was better than using Kinetin with various concentrations (2, 3, and 5 ppm) mixed with IBA at 0.5 ppm. Generally, clones proliferation was significantly better at the lower salt concentration (8000 ppm) compared with the higher ones. The same trend has been noticed concerning the interaction between growth regulators effect and salt as BA significantly improves callus proliferation with value of 5.31 proliferated clones/ jar.

**Table (4): Callus proliferation six weeks after subdividing under different salt concentrations and growth regulator treatments of *Hyoscyamus muticus* plant.**

Media type	Salt conc.			
	8000 ppm	12000 ppm	16000 ppm	Mean
MS media( control )	0.5 <sup>de</sup>	0.0 <sup>de</sup>	0.0 <sup>e</sup>	0.33 <sup>c</sup>
MS + 2.5 ppm GA + 2 ppm Ca Pantothenic acid + 1.5 ppm BA	0.31 <sup>a</sup>	2.93 <sup>b</sup>	0.0 <sup>e</sup>	2.70 <sup>a</sup>
MS + 0.5 ppm IBA + 2 ppm Kinetin	1.69 <sup>c</sup>	1.81 <sup>c</sup>	0.0 <sup>e</sup>	1.16 <sup>b</sup>
MS + 0.5 ppm IBA + 3 ppm Kinetin	1.06 <sup>cd</sup>	0.81 <sup>cde</sup>	0.0 <sup>e</sup>	0.72 <sup>c</sup>
MS + 0.5 ppm IBA + 5 ppm Kinetin	3.13 <sup>b</sup>	1.79 <sup>c</sup>	0.0 <sup>e</sup>	1.70 <sup>b</sup>
Mean	2.33 <sup>a</sup>	1.00 <sup>b</sup>	0.0 <sup>c</sup>	

**- Vegetative growth**

To evaluate the effect of two types of culture media on vegetative growth, several parameters were measured after six weeks of culture (Table

5). Data obtained revealed that culture media containing IBA at 0.5 ppm combined with 0.12 g/L  $\text{KH}_2\text{PO}_4$  + 4 ppm kinetin showed better results of 2 cm plantlets, number of new shoots/plant, shoot length, average number of leaves and plant fresh and dry weights compared with the other medium contains NAA at 0.5 ppm with the same media composition at the same lower salt level (6000 ppm).

It is noticeable that IBA was significantly better than NAA concerning most of estimated vegetative parameters. Number of new branches/ plantlet, shoot length/ cm, average leaf number/ jar, plantlet fresh weight/ g and plantlet dry weight/g have been significantly improved by using 0.5 ppm IBA with values of 2.70, 4.22, 9.97, 4.55, and 1.02 , respectively. Despite the advantage of using IBA over NAA, the last growth regulator significantly improves number of 2 cm. plantlets (11.03 and 10.07 plantlets with 6000 and 8000 ppm of salts) compared with IBA (7.53 plantlets at 6000 ppm of salts).

These results are well understood in respect of effect of growth regulators and their role in controlling shoot formation ( Torres, 1989).

#### **- Root growth**

Table (6) show that Henbane root growth was significantly better at the lower salt concentration (6000 ppm) compared with the higher one (8000 ppm).

The medium containing half strength of MS basal salts with 1 ppm of NAA was the best for root growth under the lower concentration of salts (6000 ppm).

Although of using of MS media at its half strength significantly improves henbane root growth, the medium containing salt needed to be amended with growth regulators for better root growth.

Using of half strength MS media plus NAA or IBA at 1 ppm each significantly improved root growth with the media contained 6000 ppm of salts.

These results get a long with those of Lorz *et al* (1979). It could be the lower levels of nitrogenous salts in media suitable for root formation especially with the perennial woody plants.

#### **- Plant survival and their alkaloid content**

Table (7) indicates that acclimatization of henbane plants selected under different salt concentration over a long period could be achieved. Clone A (selected at 6000 ppm of salts) and clone B (selected at 8000 ppm of salts) showed highly significant values of survival plants (28.00 and 32.02 %, respectively) compared with control plants.

However, the total percentage of alkaloids decreased for both clones compared with the control treatment.







**Table (7): Survival plants and alkaloids percentages of acclimatized clones of *Hyoscyamus muticus*.**

Clone	Survival percentage	Alkaloids percentage
control	٢٢,١١ b	١,١٠
Clone 1 ( selected at 6000 ppm)	٢٨,٠٠ a	٠,٩٠
Clone 2 ( Selected at 8000 ppm)	٣٢,٢٤ a	٠,٩٨

Generally, in most plants propagated *in vitro*, success depends on proper explants selection, medium composition, and suitable procedure for transplanting and acclimatization.

Hence, the survival plants percentages were higher despite of the salt levels may be because of using special medium containing different types of growth regulators over a long period of time (Murashige, 1977).

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## انتخاب سلالات متحملة للملوحة من بعض النباتات الطبية من خلال تقنية زراعة الأنسجة

### ١- نبات السكران المصري *Hyoscyamus muticus*

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تمت دراسة استجابة كالس نبات السكران المصري *Hyoscyamus muticus* L. لمستويات مختلفة من الملوحة. تم انتخاب سلالتين متحملة للملوحة من الكالس المنزرع على بيئة تحتوى على ٦٠٠٠ و ٨٠٠٠ جزء في المليون من الأملاح على التوالي. السلالات المنتخبة كانت قادرة على النمو و التكشف على بيئات تحتوى على نفس تركيزات الأملاح و مضاف إليها ١,٥ جزء في المليون من البنزويل أدنين. عموما الخصائص الخضريه للنباتات الناتجة ارتفعت بدرجة معنوية مع التركيزات المنخفضة من الملوحة (٦٠٠٠ ppm) خاصة على البيئات المحتوية على ٠,١٢ جرام/ لتر  $KH_2PO_4$  + ٤ جزء في المليون كينتين + ٠,٥ جزء في المليون IBA. استخدام بيئات تحتوى على نصف قوة أملاح بيئة MS مضافا إليها ١ جزء في المليون NAA كانت أفضل بيئة لنمو جذور السكران تحت مستوى ملوحة ٦٠٠٠ جزء في المليون. وصلت نسبة نجاح الشتلات المؤقتة ٢٨% للسلالة المنتخبة على ٦٠٠٠ ppm و ٣٢,٩٩٠ و لالة المنتخبة على ٨٠٠٠ ppm مع نسبة فلويدات كلية وصلت إلى ٠,٩٠ و ٠,٩٨% على التوالي.

**Table (3): Callus growth under different salt concentrations and growth regulator treatments of *Hyoscyamus muticus* plant.**

Salt Conc.	8000 ppm			12000 ppm			16000 ppm		
	No of clones / jar	Callus fresh weight/ g	Callus dry weight / g	No of clones / jar	Callus Fresh Weight / g	Callus dry weight / g	No of clones / jar	Callus fresh weight/ g	Callus dry weight/ g
<b>Media type.</b>									
<b>Control ( MS media)</b>	0.50 <sup>de</sup>	3.12 <sup>b</sup>	0.52 <sup>e</sup>	0.50 <sup>de</sup>	3.02 <sup>b</sup>	0.77 <sup>cde</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
<b>MS + 2.5 GA + 2 ppm Ca Pantothenic acid + 0.5 ppm BA</b>	3.75 <sup>a</sup>	11.12 <sup>a</sup>	2.85 <sup>a</sup>	3.50 <sup>a</sup>	1.75 <sup>b</sup>	0.06 <sup>e</sup>	0.75 <sup>cde</sup>	4.37 <sup>b</sup>	1.57 <sup>abcd</sup>
<b>MS + 2.5 GA + 2 ppm Ca Pantothenic acid + 1 ppm BA</b>	2.25 <sup>b</sup>	10.27 <sup>a</sup>	2.32 <sup>ab</sup>	3.75 <sup>a</sup>	9.90 <sup>a</sup>	1.50 <sup>bcd</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
<b>MS + 2.5 GA + 2 ppm CaPantothenic acid + 1.5 ppm BA</b>	1.50 <sup>bcd</sup>	13.27 <sup>a</sup>	2.67 <sup>ab</sup>	1.25 <sup>bc</sup>	11.22 <sup>a</sup>	1.97 <sup>abc</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
<b>Mean</b>	2.00 <sup>a</sup>	9.45 <sup>a</sup>	2.09 <sup>a</sup>	2.37 <sup>a</sup>	6.47 <sup>b</sup>	1.07 <sup>b</sup>	0.18 <sup>b</sup>	1.09 <sup>c</sup>	0.39 <sup>c</sup>

Table (5): Vegetative growth of *Hyoscyamus muticus* plant as affected by different media types and salt concentration treatments.

Salt conc.	6000 ppm						8000 ppm					
	Vegetative growth parameters						Vegetative growth parameters					
	No. of 2 cm plantlets	No. of new branches /plant	Shoot length /cm	Average leaf No. /jar	Plant fresh weight /g	Plant dry weight /g	No. of 2 cm plantlets	No. of new branches /plant	Shoot length /cm	Average leaf No. /jar	Plant fresh weight /g	Plant dry weight /g
Media type												
Control (MS media)	1.87 <sup>c</sup>	1.97 <sup>ab</sup>	2.57 <sup>b</sup>	6.42 <sup>b</sup>	3.27 <sup>b</sup>	0.60 <sup>bc</sup>	1.25 <sup>c</sup>	2.15 <sup>ab</sup>	1.55 <sup>c</sup>	5.10 <sup>b</sup>	2.22 <sup>c</sup>	0.60 <sup>bc</sup>
Control media + 0.12 g/L KH <sub>2</sub> PO <sub>4</sub> + 4 ppm Kinetin +0.5 ppm IBA	7.53 <sup>b</sup>	2.70 <sup>a</sup>	4.22 <sup>a</sup>	9.97 <sup>a</sup>	4.55 <sup>a</sup>	1.02 <sup>a</sup>	6.56 <sup>b</sup>	2.40 <sup>ab</sup>	2.15 <sup>bc</sup>	9.20 <sup>a</sup>	3.70 <sup>ab</sup>	0.95 <sup>ab</sup>
Control media + 0.12 g/L KH <sub>2</sub> PO <sub>4</sub> +4 ppm Kinetin + 0.5 ppm NAA	11.03 <sup>a</sup>	1.55 <sup>b</sup>	2.02 <sup>bc</sup>	2.92 <sup>c</sup>	2.92 <sup>c</sup>	0.16 <sup>d</sup>	10.07 <sup>a</sup>	0.37 <sup>c</sup>	1.82 <sup>bc</sup>	2.97 <sup>c</sup>	2.97 <sup>c</sup>	0.25 <sup>cd</sup>

Table (6): Root growth of *Hyoscyamus muticus* plant as affected by different media types and salt concentration treatments.

Salt conc.	6000 ppm				8000 ppm			
	Root growth parameters				Root growth parameters			
	Average No. of roots	Average length of roots / cm	Root fresh weight /g	Root dry weight /g	Average No. of roots	Average length of roots /cm	Root fresh weight /g	Root dry weight /g
Media type								
Control ( MS media)	5.82 <sup>de</sup>	2.10 <sup>d</sup>	4.05 <sup>e</sup>	0.06 <sup>d</sup>	9.15 <sup>cd</sup>	6.12 <sup>abc</sup>	7.15 <sup>cd</sup>	0.75 <sup>b</sup>
MS media half strength	4.05 <sup>e</sup>	0.82 <sup>d</sup>	4.72 <sup>e</sup>	0.11 <sup>d</sup>	8.27 <sup>cd</sup>	4.77 <sup>c</sup>	5.22 <sup>de</sup>	0.57 <sup>bcd</sup>
MS media half strength+ 1 ppm NAA	15.75 <sup>a</sup>	7.67 <sup>a</sup>	11.30 <sup>a</sup>	1.85 <sup>a</sup>	8.92 <sup>cd</sup>	7.22 <sup>a</sup>	5.57 <sup>de</sup>	0.20 <sup>cd</sup>
MS media half strength+ 1 ppm IBA	16.05 <sup>a</sup>	7.77 <sup>a</sup>	9.60 <sup>a</sup>	1.00 <sup>b</sup>	10.15 <sup>bc</sup>	4.47 <sup>c</sup>	3.55 <sup>e</sup>	0.67 <sup>bc</sup>
MS media half strength+ 5 ppm NAA	11.40 <sup>bc</sup>	5.45 <sup>bc</sup>	7.10 <sup>cd</sup>	1.00 <sup>b</sup>	15.72 <sup>ab</sup>	6.65 <sup>ab</sup>	8.80 <sup>bc</sup>	0.82 <sup>b</sup>
MS media half strength+ 5 ppm IBA	7.52 <sup>cde</sup>	4.32 <sup>c</sup>	5.40 <sup>de</sup>	0.65 <sup>bc</sup>	13.72 <sup>ab</sup>	5.02 <sup>bc</sup>	7.30 <sup>cd</sup>	0.75 <sup>b</sup>