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## Utilization of Aquatic Plants Extracts as an Alternative to Plant Growth Regulators In Vitro Experiments

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### ABSTRACT

In the current study, the levels of endogenous free and conjugated auxin (Indole-3-acetic acid, IAA), gibberellic acid (GA<sub>3</sub>), and abscisic acid (ABA) were examined in two species of aquatic plants (*Ceratophyllum demersum* and *Egeria densa*). The comparison between the content of endogenous phytohormones in aquatic plants showed that *C. demersum* had highest levels of total (IAA, GA<sub>3</sub> and ABA) than *E. densa*. Different concentrations of free phytohormones extracts were prepared (0, 25, 50, and 100 μl/l), then added to (MS) culture medium as an alternative to plant growth regulators. Effects of these concentrations on callus production of black henbane (*Hyoscyamus niger*) and potato propagation (*Solanum tuberosum*) were studied *in vitro*. Free phytohormones extracts with all concentrations encouraged production of callus tissue from leaves explant of black henbane compared with control treatment, also, these extracts promoted propagation of potato by increasing number of nodules and length of shoot. So, it appears that remnants of aquatic plants could be used successfully for agricultural improvement and another application of bioassay.

### INTRODUCTION

Plant growth hormones (Indole-3-acetic acid IAA, Gibberellic acid GA<sub>3</sub> and Abscisic acid ABA) are specialized chemical substances or known as secondary metabolites, which are important biotechnological products, widely used in agricultural and horticultural applications.

These hormones are synthesized not only by higher plants, they have also been synthesized by Mosses, Lichens (Ergün *et al.*, 2002), Fungi (Ünyayar *et al.*, 1996; MacMillan, 2002; Rangaswamy, 2012), Bacteria (Karadeniz *et al.*, 2006), Yeast (Tawfiq, 2010) and Algae (Jacobs *et al.*, 1985). Hormones are synthesized in plant material at a very low concentration, together with many other compounds (Muller, 2011; Karadeniz *et al.*, 2006) for this reason, be high cost in the market.

In this respect, were chosen two types of aquatic plants (*Ceratophyllum demersum* and *Egeria densa*) to estimate the content of IAA, GA<sub>3</sub> and ABA in their tissues and use it as substitute for plant growth regulators in plant tissue culture experience for employ cheap raw materials to rendering it economical.

*Ceratophyllum demersum* (hornwort) is a submersed member of the Ceratophyllaceae family and *Egeria densa* (Elodea) belonging to the family

Hydrocharitaceae. Both species have a similar distribution patterns in Iraq, they are widespread, Submersed and abundant plants (Al-Daody and Al-Mandeel, 2012; Bowmer *et al.*, 1995; Aziz, 2009), can be found in sluggish lakes, ponds, and slow streams (Schmidt and Kannenburg, 1998).

Most of waterweed poses serious environmental problems when the dense growths are over 25% of the surface area. Aquatic plants can restrict swimming, boating, fishing and reduce water flow to irrigated regions. These plants provide a food source for many freshwater organisms, including aquatic insects, fish and aquatic invertebrates, plus help to stabilize bottom sediments (Helfrich *et al.*, 2000).

Aquatic plants like *C. demersum* and *E. densa* can be used as biofilter for heavy metals such as Ni, Pb, and Cd (Módenes, 2009; Foroughi *et al.*, 2011; Dhir, 2013). Many countries are currently utilizing aquatic plants for agricultural purposes as food for cattle and sheep, poultry, and as preparation for silage, because they are rich in protein, minerals and pigments carotenoids (Edwards, 1981).

The aim of our investigation was to determine IAA, GA<sub>3</sub> and ABA in the aquatic plants and use it as alternative to plant growth regulators which are widely used in plant tissue culture technique and many areas of horticulture including pomology and ornamental horticulture.

## MATERIALS AND METHODS

### Collection of samples

Two different samples of common Iraqi aquatic plants (*C. demersum* and *E. densa*) were collected on March and April in 2014 from streamlet of Baghdad University, placed in plastic bags, and transferred immediately to the laboratory. Each plant was washed with fresh tap water and cleaned to remove impurities and then oven-dried at 45°C for 48 h.

### Extraction of plant growth regulators IAA, GA<sub>3</sub> and ABA

One gram of dry tissue per sample was homogenized for 24 h with 60 ml of mixture of solvents (methanol: chloroform: 2N ammonium hydroxide, 12:5:3 v/v/v) at 4°C. Both combined extract (60 ml) was centrifuged for 15 min at 2500 rpm/min, the supernatant was treated with 25 ml of distilled water. The other steps of free and bound hormones extraction were done according to Kelen *et al.*, 2004. The dried residues were dissolved in 10 ml of 70% methanol, then the Optima UV Spectrophotometer (Optima SP-3000 nano, Japan) was used to estimate the content of free and conjugate forms of phytohormones using 222 nm and 280 nm wave lengths for IAA, 254 nm for GA<sub>3</sub> and 263 nm for ABA. IAA standard reagent was obtained from Sigma Chemical Co. (USA), GA<sub>3</sub> from Merck Co. (German) and ABA from Himedia Co. (India).

### Phytohormones Bioassay

Free phytohormones extracts for both *C. demersum* and *E. densa* were tested *in vitro* on callus formation from leaves of *Hyoscyamus niger* (black henbane), and on propagation of potato (*Solanum tuberosum* L.). The culture medium used for all experiments as based on (MS) Murashige and Skoog's medium (Murashige and Skoog, 1962).

Leaves explant of black henbane (*H. niger*) was cultured for induction of callus using MS medium supplemented separately with 0, 25, 50 and 100 µl/l each of *C. demersum* and *E. densa* free phytohormones extracts. Fresh and dry weights of callus were recorded after 8 weeks of incubation at 25 ± 2°C under a 16 h/day photoperiod.

Furthermore, single nodules of potato explant were planted in MS medium supplemented separately with 0, 25, 50 and 100  $\mu\text{l/l}$  of free phytohormones extracts. All cultures were incubated at  $25 \pm 2^\circ\text{C}$  under 16h/day in photoperiod condition. Number of nodules and length of plantlets were recorded after 4 weeks.

### Experimental design

All experiments were done with minimum of 20 replicates per treatment. A one-way ANOVA with replication was done using Statistical Software-Minitab 11. Least significant differences (LSD) between means were calculated at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Phytohormones content

The IAA,  $\text{GA}_3$  and ABA content of two aquatic plant samples were obtained by spectrophotometric method. Calibration curves of these three substances were prepared using analytical reagent grade standards. Linear regression data for IAA,  $\text{GA}_3$  and ABA are listed in Figure 1. The comparison between two species of aquatic plants showed that *C. demersum* had highest levels of total phytohormones than *E. densa*.

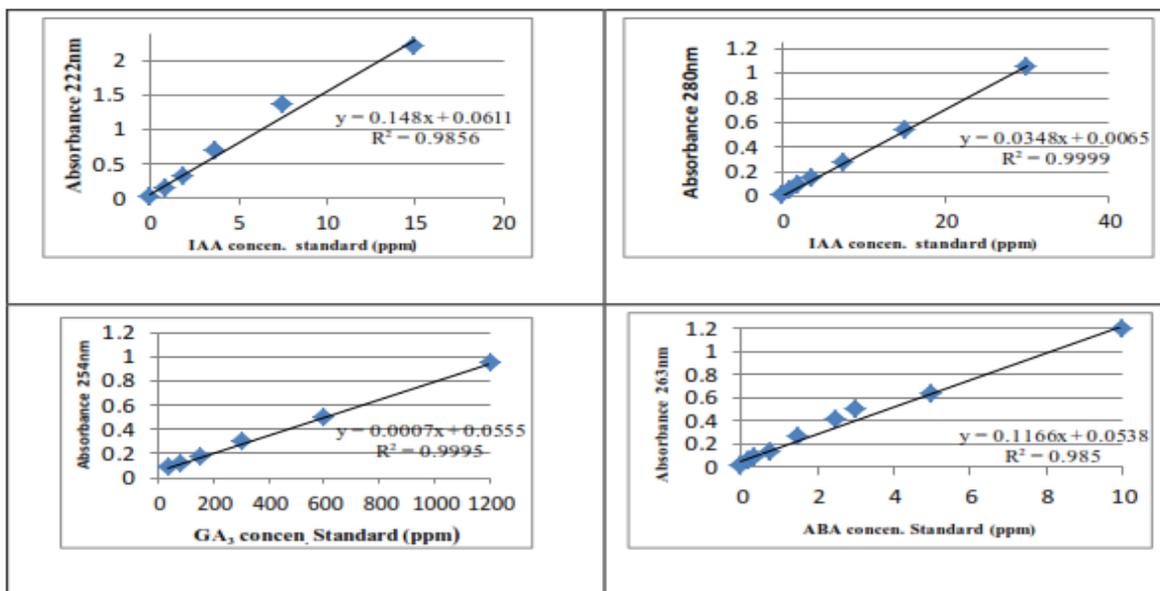


Fig. 1: The standard curves for plant growth regulators using spectrophotometric method; IAA (222 and 280 nm),  $\text{GA}_3$  (254nm) and ABA (263nm).

Total values of IAA in *C. demersum* were (53.49 and 132.17 ppm) at 222 and 280nm wavelengths respectively, while the total levels of this compound were found in *E. densa* (53.73 and 98.55 ppm) respectively. Also highest  $\text{GA}_3$  and ABA concentrations were recorded in *C. demersum* (8614.28 and 77.135 ppm) respectively, and the lowest total levels of these compounds were determined in *E. densa* (5314.28 and 30.56 ppm) respectively. The amounts of IAA,  $\text{GA}_3$  and ABA in two species of aquatic plants are given in Table (1).

Table 1: Content of phytohormones (ppm) in two species of aquatic plants (*Ceratophyllum demersum* and *Egeria densa*) using spectrophotometric method.

Phytohormones (ppm)		Aquatic plants species	
		<i>Ceratophyllum demersum</i>	<i>Egeria densa</i>
IAA 222nm	Free IAA	33.64	28.57
	Conjugate IAA	19.85	25.16
	Total IAA	53.49	53.73
IAA 280nm	Free IAA	73.99	51.86
	Conjugate IAA	58.18	46.69
	Total IAA	132.17	98.55
GA3 254 nm	Free GA3	4578.57	2050
	Conjugate GA3	4035.71	3264.28
	Total GA3	8614.28	5314.28
ABA 263 nm	Free ABA	55.506	16.74
	Conjugate ABA	21.629	13.82
	Total ABA	77.135	30.56

### Phytohormones bioassay

Bioassay test was done only on free phytohormones form because the conjugate forms are generally considered inactive (Korasick *et al.*, 2013). Effect of free phytohormones extracts on fresh and dry weights of callus production from leaves of black henbane (*H. niger*) was studied. Generally, the enhancement of mass callus production depended on the type plant extract and their concentration. The result shows that all treatments of phytohormones extract significantly encouraged the induction of callus comparison with control.

*C. demersum* extract enhanced the fresh and dry weights of callus tissue more than *E. densa*, (Table 2). Furthermore, these phytohormones extracts promoted *in vitro* propagation of potato (*S. tuberosum*) by raising number of nodes more than control treatment. The results also showed the presence of significant differences in length of plant produced depending on the type of aquatic plant extract and their concentration, (Table 3).

Table 2: Beneficial effects of free phytohormones extracts added to MS medium on fresh and dry weights (mg) of callus production from leaves of (*Hyoscyamus niger*) after 8 weeks of incubation.

Amount of phytohormones extracts	<i>Ceratophyllum demersum</i>		<i>Egeria densa</i>	
	Fresh weight of callus	Dry weight of callus	Fresh weight of callus	Dry weight of callus
0.0(control)	—	—	—	—
25 µl/l of	1200.0	90.1	1000.5	76.36
50 µl/l of	1281.7	93.6	941.3	68.93
100 µl/l of	1280.8	99.2	893.5	69.97
Means	940.6	70.7	708.8	53.8
LSD at P ≤ 0.05	69.50	4.11	40.7	2.96

Table 3: Beneficial effects of free phytohormones extracts added to MS medium on potato (*Solanum tuberosum*) propagation after 4 weeks of incubation.

Amount of phytohormones extracts	<i>Ceratophyllum demersum</i>		<i>Egeria densa</i>	
	Number of nodes	Length of plant (cm)	Number of nodes	Length of plant (cm)
0.0 (control)	5.3	4.2	5.3	4.5
25 µl/l	7.0	6.1	6.3	4.5
50 µl/l	7.0	7.0	7.3	5.5
100 µl/l	6.5	6.2	7.6	6.0
Means	6.4	5.8	6.6	5.1
LSD at P ≤ 0.05	0.4	0.4	0.5	0.3

## DISCUSSION

The comparison between the content of endogenous phytohormones in *C. demersum* and *E. densa* show variation at the levels of phytohormones between them. It is worth mention that the endogenous levels of several phytohormones in aquatic macrophytes are variable during the annual cycle of the plants (Best, 1982). Aquatic macrophytes are plants that have adapted to living in watery environments. In spite of the numerous environmental problems caused by these plants, but there are many solutions that make these plants economically beneficial. Of these solutions is used as feed for poultry and livestock (Jassim *et al.*, 2006; Easley and Shirley, 1974), and use it as fertilizer to increase the fertility of the land or to enhance the growth of plants, because several authors reported that aquatic plants considered as perfect sources for ash, proteins and other organic materials (Edwards, 1981).

In presented study, greater effectiveness of medium supplemented with aquatic plant extracts than un supplemented medium in promoting callus formation of black henbane and enhancing growth of potato could be explained that these plants have a good amounts of plant hormones. Thus, the addition of aquatic plant extracts to culture media is beneficial and suitable measure to improve *in vitro* culture media which used for commercial production. There are several reports on successful using plant extracts as an alternative to plant growth regulators.

One of these report is that of Ibrahim *et al.*, 2008, who succeeded in growing soya bean, potato and wheat plants in *in vitro* culture by adding the *Glycyrrhiza glabra* callus extracts to their culture medium as an alternative to plant growth regulators. Also, occurrence of auxin and gibberellin-like substances in coconut milk have been reported by (Dix and Van Staden, 1981). So, many researchers found that coconut milk could be used to initiate and induce the growth *in vitro* plant tissue cultures (George, 200; Thorpe *et al.*, 2008). A wide variety of organic extracts are now commonly added to culture media and are often reported to promote growth, these include tomato juice, ground banana, orange juice, carrot juice, malt extract, yeast extract, leaf extracts, casein hydrolysate, (Puchooa and Ramburn, 2004; Dodds and Roberts, 1985; Saad and Elshahed, 2012). likewise, Straus (Straus, 1960) has shown that complex organic extracts function by supplying a form of organic nitrogen content (a mixture of amino acids).

In our study, the Analysis of phytohormones showed that IAA, GA<sub>3</sub> and ABA are produced with good amounts in aquatic plants. So, it is believed that success of using these plants as an alternative to plant growth regulators could be reducing the cost of *in vitro* plant tissue cultures, or can be used as green manure or biofertilizers to add phytohormones and organic matters to the soil.

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