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Detection and Quantification of Auxin and Gibberellic Acid in *Caulerpa racemosa*

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Abstract This study was conducted to evaluate plant growth regulators (PGRs) presented in extract of seaweed, *Caulerpa racemosa* by Spectrophotometry. Spectra were recorded at the m/z peak of 220 nm for Auxin, and at 524 nm m/z peak for Gibberellic acid in seaweed extract. Extraction, purification and quantitative determination of free and bound NAA, and GA3, in *C. Racemosa* were done by reducing 250 mL seaweed supernatant to 50 mL by evaporation and acidified to pH 2.8 with 1 N HCl and extracted with double volume of ethyl acetate. On the otherhand, GA3 was extracted and purified. Fifty mL of cell free supernatant was reduced to 20 mL by evaporation and acidified at pH 2 and then was extracted with double volume of chloroform. The supernatant was harvested by centrifugation and reduced to 30 mL by evaporation under vacuum. All experiments were repeated three times. Statistical analysis performed using SPSS statistical software (SPSS Inc., USA) for correlation and regression analysis of each value. The extract contained 979.71 mg/l of GA3 and 15.57 mg/l of NAA at 10 ppm concentration respectively. The results confirmed the presence of plant growth regulators (PGRs) (NAA, and GA_3).

Keywords: Seaweed, Plant Growth Regulators, Auxin (NAA), Gibberellic Acid (GA3), Spectrophotometry Technique

Introduction

Fast growth rate in plant species maybe indicative of presence of growth regulators. Aside from higher plants, fungi and bacteria, there are evidences that algae also synthesize plant growth regulators auxin, gibberellic acid, abscisic acid and cytokinin (Zodape *et al*., 2010).

Seaweeds are rich sources of growth promoting substances (Sylvia *et al.*, 2005) such as NAA, kinetin, zeatin and gibberellins (Zodape *et al.*, 2010) auxins and cytokinins (Zhang and Ervin, 2004); metabolic enhancers (Zhang and Schmidt, 1997) ; macro and micro elements (Strik *et al.*, 2003), amino acids, vitamins and beneficial results from their use in crop plants like early

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seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress and enhanced post-harvest shelf life of seeds (Hankins and Hockey, 1990; Blunden, 1994).

The benefits of seaweeds as sources of organic matter and fertilizer nutrients have led to their use as soil conditioners for centuries. Seaweed components such as macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid (ABA)-like growth substances affect cellular metabolism in treated plants leading to enhanced growth and crop yield (Crouch *et al*., 1993; Crouch and van Staden, 1993a; Reitz and Trumble, 1996; Duran *et al*., 2003; Stirk *et al*., 2003; Gandhiappan *et al*., 2004). Seaweed extracts are bioactive at low concentrations (diluted as 1:1000 or more). Although many of the various chemical components of seaweed extracts and their modes of action remain unknown.

Algae specifically *C. racemosa* belong to a very large and diverse group of simple, typically autotrophic organisms synthesized phytohormones. Their functions, synthesis and effects, are of great interest (Proseus *et al*., 2006). To study them in plant tissues, accurate and sensitive methods are required. Although the full-value of plant hormone systems in algae is still under debate, the aforementioned studies about auxins on algal growth and development indicate that its functions likely correspond to its activity in higher land plants (Tarakhovskaya *et al*., 2007).

There are a limited number of reports on the level, metabolism and physiological effects of all of these plant growth regulators in seaweed. However, almost no study has been directed toward the level of production, metabolism and physiological effects of all of these plant growth regulators. For this reason, the study was formulated with the level of NAA, GA3, in *C. racemosa*.

Materials and Methods

Crude Extract

Seaweed species specifically, *C. racemosa* was used as experimental material. Five hundred (500) grams of pulverized *C. racemosa* was soaked for 72 hours in 1000 ml of 80 % ethanol (1:2, 1 g of powdered leaves is to 2 ml of ethanol) for the sample extract. The obtained extract was filtered with sterilized filter paper and subjected to rotary evaporator set at 60rev/min at 45℃. The filtrate or crude extract obtained was stored in amber bottles and kept refrigerated until use.

Extraction

Extraction, purification and quantitative determination of free and bound NAA, and GA3 in *C. racemosa* were done, according to the methods of Unyayar *et al*. (1996). The extraction and purification procedures were showed in Fig. 1. Spectrophotometric techniques was used to determine the amounts of NAA, and GA3, a total of 500 gram dry weight of each sample was taken and combined with 1000 ml of ethanol. Each combined extract were kept in a bottle at -20˚C in deep freeze for further analysis. NAA, and GA3, extraction assays were done according to the schematic diagram.

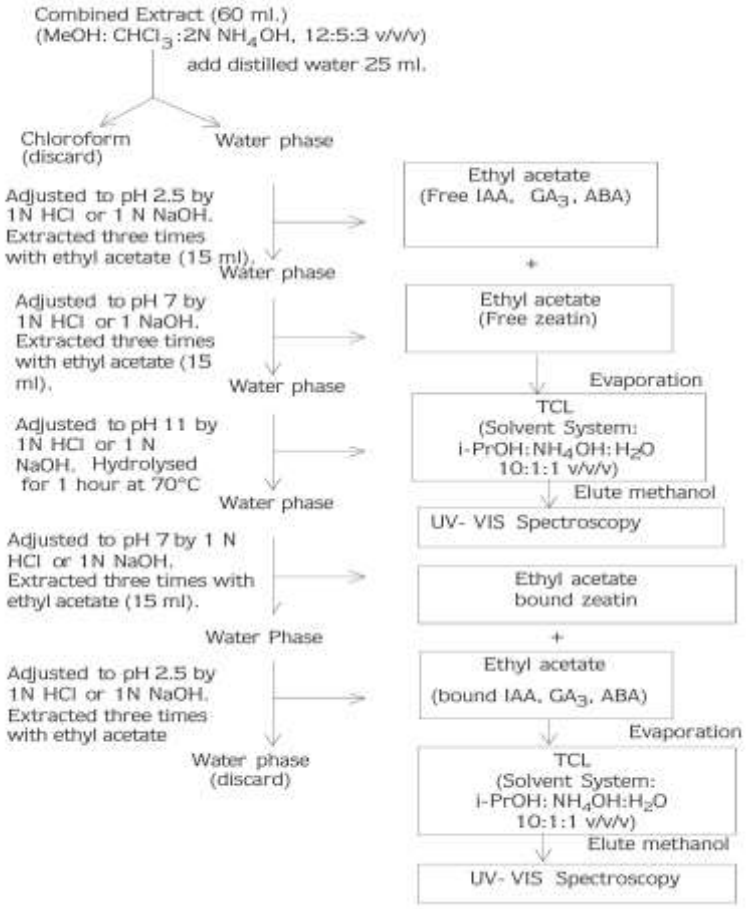

Figure 1. Extraction, purification and quantitative determination of free and bound NAA, and GA3

Extraction and Purification of NAA, and GA3

NAA was extracted and purified following the method described by Unyayar *et al*. (1996) with some modifications. The 250 mL seaweed supernatant was reduced to 50 mL by evaporation and acidified to pH 2.8 with 1 N HCl and extracted with double volume of ethyl acetate. Besides, GA3 was extracted and purified following the method described by Shanmugam and Narayanasamy (2009). For extraction and purification of GA production, 50 mL of cell free supernatant was reduced to 20 mL by evaporation and acidified at pH 2 and extracted with double volume of chloroform. The supernatant (cell-free liquid culture medium) was harvested by centrifugation at 8000 rpm for 15 min at 4 ℃ and reduced to 30 mL by evaporation under vacuum.

Spectrophotometry Assay

Assay was done by using 224 nm wave lengths for NAA, 254 nm for GA3, and for all standard synthetic NAA, and GA3, isolated samples. Chemicals and standards. Standard Napthalene acetic acid (NAA), Gibberellin (GA3) ($C_{19}H_{22}O_6$, MW 346.38) were purchased from RTC laboratory service and supply.

All experiments were repeated three times. Total NAA, and GA3, were obtained as the sum of free and bound NAA, and GA3. The amounts of NAA, and GA3 in the samples were expressed as standard synthetic NAA, and GA3 equivalent. Statistical analysis performed using SPSS for windows statistical software (SPSS Inc., USA) for correlation and regression analysis of each value.

Results

Identification of PGRs by Spectrometry, colorimetric studies provided positive indication of the presence and concentration of auxin, and gibberellin constituents in *C. racemosa.* To prove the constituents more precisely, spectra were recorded. The m/z peak at 174 for Auxin, and 220 nm and m/z peak at 345.08 for GA3 at 524 nm for Gibberelin in seaweed extract.

Results of the correlation Analysis of absorbance rate of auxin (NAA) is shown in Figure 2. Correlation coefficient of variables R= 0.9911 represented very strong positive correlation indicated that the level of auxin increases as the level of concentration of the extract increases. Regression analysis indicated that the average auxin level of seaweed extract was y= 0.0036, if additional concentration is not added. The concentration of auxin in the extract, on the basis of the analysis of the ethanol extract, was estimated to be 15.57 mg/l (10 ppm).

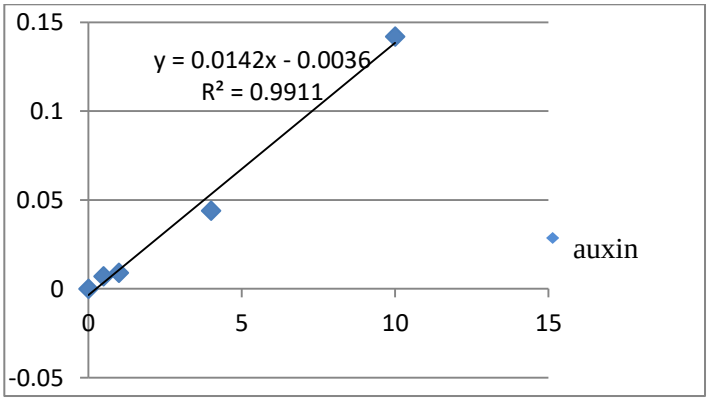

Figure 2. Correlation and Regression analysis of absorbance rate of Auxin (NAA).

The Correlation and Regression analysis of absorbance rate of gibberelic acid (GA3) showed in Fig. 3. For quantification of GA3, a calibration plot was first made of GA3 with R2 = 0.98, indicating a very strong positive correlation of GA3 and concentration variables. Regression analysis indicated that the average gibberelin level of seaweed extract was y= 3.43 if additional concentration is not added. The concentration of GA3 in the extract, on the basis of the analysis of the ethanol extract, was estimated to be 979.71 mg/l (10 ppm.)

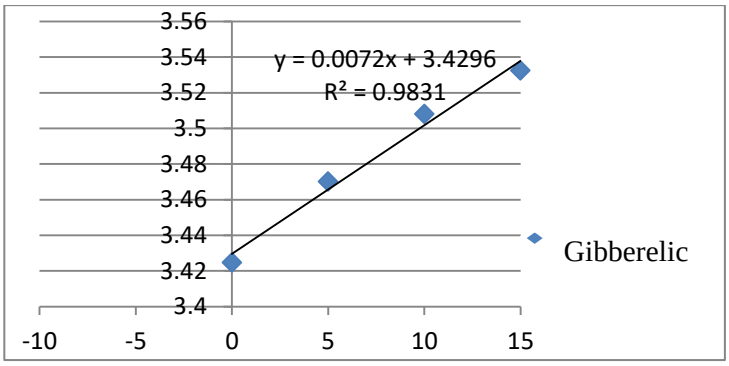

Figure 3. Correlation and Regression analysis of absorbance rate of Gibberelic acid (GA3)

Discussion

Identification of PGRs by Spectrometry, colorimetric studies provided positive indication of the presence and concentration of auxin, and gibberellin

constituents in the extract. To prove the constituents more precisely, spectra were recorded. The m/z peak at 174 for Auxin, respectively 220 nm and m/z peak at 345.08 for GA3 at 524 nm for Gibberelin in seaweed extract.

For quantification of NAA and GA3, a calibration plot was first made of GA3 with R2 = 0.98 and for NAA calibration plot with R2 = 0.99. This indicates a very strong positive correlation of NAA, GA3 and concentration variables. Regression analysis indicated that the average auxin and gibberelin level of seaweed extract were y= 3.43 and 0.0036 if additional concentration is not added. As mentioned, when the laser excitation energy is closed to different constituent molecules in algae with respect to the excitation wavelength (532 nm) molecule under investigation, resonant scattering would occur (Spiro, 1994).

The results from the above experiments carried out with the extract of *C. racemosa* confirmed the development of an easy and accurate method of quantification of PGRs (NAA, and GA3) using fragmentation patterns for the standards that are provided in the supporting information. Due to chemical diversity of the phytohormones, as a result of their substituent groups on the one hand and due to the chemical similarity of these substances within a particular group, it was necessary to optimize several experimental conditions (wavelength, column, temperature, mobile phase) for their sensitive and effective simultaneous detection (Nordstrom *et al*., 2004). These results indicated good agreement with reported data. It can be observed from the above data that auxin was detected in the extract in small amounts estimated to be 15.57 mg/l (10 ppm), although it is usually considered to be a natural substance. In normal conditions, scattering intensity of an active component depends on the incident laser frequency (Smith and Dent, 2005). The intensity of a characteristic peak also scales linearly with the concentration of the molecule which produces the spectrum. The absorption maxima of the each phytohormone are different.

Occurrence of growth regulators and their effect alter the physiological attributes (Temple and Bomke, 1989) and presence of growth promoting substances including auxins and gibberellins in seaweed (Zhag *et al*., 1997) have been reported. The phytohormone auxin is one of the main directors of plant growth and development. In higher plants, auxin is generated in apical plant parts and transported from cell-to-cell in a polar fashion (Fujita *et al*., 2009). However, Bentley-Mowat and Reid (1999) have previously reported the presence of NAA like substances in algae in small amount, Furthermore, in our studies the mass fragmentation was found to be identical to that of an authentic sample of NAA and also tallied with the mass spectra available in the database. As NAA is responsible for cell division, increased in growth parameters,

stimulates the occurrence of adventitious roots and cell division, concentration differences of auxin can cause different responses in plant development (Dumale *et al*., 2016).

The large amount of gibberellin detected in concentration of GA3 in the extract, on the basis of the analysis of the ethanol extract, was estimated to be 979.71 mg/l (10 ppm.), Gibberelin hormones known responsible for growth and cell elongation of plants, GA3 promotes increase germination by 6.5%, length and leaf blade length (Dumale *et al*., 2016). Studies in the large coenocytic green alga *Caulerpa paspaloides* show same results. At low concentrations, they stimulate the growth and affect the development, cell division, cell elongation and vascular differentiation but have inhibitory effect on root growth (Fujioka & Sakurai, 1997).

Conclusion

This study was conducted to identify PGRs by Spectrometry, colorimetric studies for positive indication of the presence and concentration of auxin, and gibberellin constituents in the seaweed extract of *C. racemosa*. To prove the constituents more precisely, spectra are recorded, the m/z peak for auxin is 220 nm and m/z peak for GA3 at 524 nm for gibberelin in seaweed extract. The extract of *C. racemosa* confirm the presence of method of quanti fication of a few PGRs (NAA, and GA_3) using spectrometry method. The extract found to contain 979.71 mg/l of GA3 at 10 ppm and 15.57 mg/l at 10 ppm concentration of NAA. The concentrations obtained from the extract gibberellic acid is higher and lower in Auxin cases.

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