Determination of plant growth regulators (Abscisic acid, Indole-3-butyric acid, Zeatin riboside) in liquid seaweed fertilizers by immunoassay

Byungchul Kim, Jingping Xie and Titan Fan

Beacon Analytical Systems, Inc., Saco, ME, USA

Abstract

Plant growth regulators such as abscisic acid (ABA), indole-3-butyric acid (IBA), zeatin riboside (ZR) have been found in terrestrial plants as well as seaweeds. The seaweed extracts have been commercially used as growth stimulant (fertilizer as soil conditioner) in agriculture and horticulture for many years. However, the levels of those components are not usually indicated on the product labels. Therefore, simple and easy immunoassays for the detection of abscisic acid, indole-3-butyric acid, zeatin riboside were developed. Rabbit polyclonal antibodies were produced after immunization and used for the development of assays. Assay time for each analysis was 70 minutes. The detection limit for both abscisic acid and indole-3-butyric acid was 10 ppb. Assay for the zeatin riboside had a sensitivity of 0.3 ppb in buffer solution, and an assay range of 0.3 to 30 ppb. Assay range for abscisic acid and indole-3-butyric acid was 10 to 500 ppb in buffer solution. Using those three separate immunoassays, eight commercial seaweed extracts were analyzed and compared. Ranges of ABA, IBA and ZR determined in the commercial products were 0.04 – 14.8 ppm, 0.13 – 30.7 ppm and 0.01 – 1.08 ppm, respectively.

Introduction

Seaweed extracts have been used in agriculture to improve growth and yield of crops such as soybean (Rathore et al., 2009), potato (Blunden et al., 1977), pepper (Eris et al., 1995), sugar beet (Blunden et al., 2009) and okra (Zodape et al., 2008). There are many seaweed extract manufacturers from the east coast of the United States. In the state of Maine, seaweed (rockweed) landings in 2010 were 10.7 million pounds which is worth more than $253,000. More landings are expected because more organic seaweed extracts will be demanded by farmers to replace chemical fertilizers due to high demands for organic produce. The harvested seaweed is processed and used in fertilizer. It is believed that a number of plant growth-stimulating compounds in the seaweed such as abscisic acid (ABA), indole-3-butyric acid (IBA), and zeatin riboside (ZR) may play an important role. However, most of the commercial seaweed extracts in the market don't indicate the amount of these compounds. Commercial seaweed extracts will be more attractive to customers if they can see the levels of those compounds on the label. Analytical methods such as HPLC (high performance liquid chromatography) and GC (gas chromatography) have been used to analyze these compounds. However, tedious sample clean-up procedure makes those analytical methods less attractive. Using a simple and easy immunoassay, determination of these compounds in seaweed extracts can be achieved by the manufacturers of seaweed extract products. Immunoassays will help seaweed extract manufacturers conduct good quality control for manufacturing products that contain constant levels of the compounds. In this study, competitive immunoassays were developed and used to determine ABA, IBA and ZR contents from commercial seaweed extracts.

Figure 1. Commercial seaweed extracts.

Methodology

Polyclonal antibody production:

In the development of these assays, Abscisic acid, Indole-3-butyric acid or Zeatin riboside was coupled to a modified protein carrier, which served as an immunogen, and horse radish peroxidase (HRP) which served as the enzyme conjugate. The antibody was produced through the immunization of New Zealand white rabbits. The rabbits were shaved and injections were administered intradermally on the back. The serum was collected monthly.

Sample preparation:

Weigh 0.2g of seaweed extract and mix with 20 mL of 10% MeOH in PBS buffer (pH 7.4).
Dilution is necessary with 10% MeOH in PBS buffer (pH 7.4) when OD value is out of range.
Filter the diluted extract with filter paper if visible debris observed.

Assay protocol:

50 µl of HRP conjugate is added to each well followed by 50 µl of calibrator or sample extract. Lastly 50 µl of antibody solution is added to the wells and mixed gently for 30 seconds and incubated for 30 minutes. The well contents are then decanted and washed 4 times with PBST wash solution. Substrate (100 µl) is added to the wells and incubated for 30 minutes. The reaction is stopped with 100 µl of Stop solution and the contents of the wells are read at 450nm-650nm. The concentration is calculated from a calibration curve applying dilution factors.

Calibrators for each assay:

- ABA: 0, 10, 100 and 1000 ppb
- IBA: 0, 10, 100 and 1000 ppb
- ZR: 0, 1, 10 and 100 ppb

Table 1. Detection range for each assay.

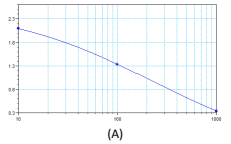
Assay	Detection range
Abscisic acid	10 – 500 ppb
Indole-3-butyric acid	10 – 500 ppb
Zeatin riboside	0.3 – 30 ppb

Results

Table 2. Comparison of commercial products for ABA, IBA and ZR contents.

Sample	Organic	ABA (µg/g, ppm)	IBA (µg/g, ppm)	ZR (µg/g, ppm)	ABA+IBA+ZR/$
1	Organic	0.04	0.13	0.01	24 ppm/$
2	Nonorganic	6.78	7.73	0.09	750 ppm/$
3	Organic	0.07	0.14	0.01	4 ppm/$
4	Organic	1.73	1.24	0.04	285 ppm/$
5	Natural	0.05	0.23	0.01	7 ppm/$
6	Organic	14.8	4.74	0.22	575 ppm/$
7	Natural	0.06	0.25	0.01	5 ppm/$
8*	Nonorganic	0.07	30.7	1.08	1662 ppm/$

*No information on the product label. Stated as "It is packed with a blend of cytokinins, auxins and Gibberellic acid".



(A)

4-parameter curve fit: Y = (A – D)/(1 + (X/C)^B) + D

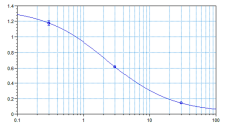
A	B	C	D	R^2
2.53	0.582	225	-0.576	1

IC 50: 116 ppb, IC 85: 8 ppb

(B)

A	B	C	D	R^2
1.42	0.963	51	0.0416	1

IC 50: 54 ppb, IC 85: 8 ppb



(C)

A	B	C	D	R^2
1.24	0.973	2.72	0.092	1

IC 50: 3.2 ppb, IC 85: 0.5 ppb

Figure. 2. Standard curves. (A) ABA, (B) IBA and (C) ZR

Discussion

Three separate immunoassays were developed to detect the concentration of Abscisic acid, Indole-3-butyric acid and Zeatin riboside in commercial seaweed extracts. Eight commercial liquid fertilizers were tested. The amount of each compound was determined, and total levels of the three compounds were also calculated based on cost of the product. Since the volume of each product is different, the amount of ingredients per dollar would be a good way to compare the products. Among organic seaweed products, the most cost effective product (sample 6) has 575 ppm of the combined compounds per dollar. The worst seaweed product (sample 3) only contains 4 ppm of the combined compounds per dollar. The difference between them is more than a hundred times. There are two nonorganic products (sample 2 and 8) that have high levels such as 750 and 1662 ppm per dollar. However, since they are not organic, it is possible that they were processed with addition of the compounds by the manufacturer. It is stated on the label of the product (sample 8) that cytokinins, auxins and gibberellic acid were added. These three immunoassays were successfully evaluated for determining the plant growth regulators such as Abscisic acid, Indole-3-butyric acid and Zeatin riboside in commercial seaweed extracts. These immunoassays can provide fast and reliable results to seaweed extract processors for quality control purposes.

References

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