Journal of Applied Phycology **8**: 503–508, 1997.

Comparison of cytokinin- and auxin-like activity in some commercially used seaweed extracts

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Received 17 August 1996; revised 20 September 1996; accepted 21 September 1996

Key words: auxin-like activity, cytokinin-like activity, mung bean bioassay, seaweed extracts, soybean callus bioassay

Abstract

Six commercially used seaweed extracts were tested for cytokinin- and auxin-like activity using the soybean callus bioassay and the mung bean rooting bioassay respectively. All the seaweed extracts tested showed cytokinin-like activity. Seamac caused the most callus growth with activity being concentrated at R_f 0.9 which co-chromatographed with *iso*-pentenyladenine and its riboside. The other five seaweed extracts yielded activity at R_f 0.7–0.8. This co-chromatographed with zeatin and zeatin riboside. All the seaweed extracts tested improved rooting of mung beans. Kelpak made from *Ecklonia maxima* (Osbeck) Papenf. gave the best rooting response.

Introduction

Seaweed extracts are used extensively in agriculture as plant growth supplements. There are numerous reports in the literature of seaweed extracts increasing crop yields, improving growth, increasing plant resistance to frost, fungal and insect attack, causing a reduction in red spider, aphid and nematode infestations, reducing storage loss in fruit and increasing inorganic nutrient uptake from the soil (see Mooney & van Staden, 1986). As seaweed extracts are applied in small dosages, it is clear that the active ingredient(s) in seaweed extracts are effective in low concentrations. The low nutrient concentration in seaweed extracts cannot adequately account for the observed beneficial plant responses. Plant growth regulators (PGR), and in particular cytokinins, have been implicated in improved growth as responses obtained with application of exogenous cytokinins were similar to those obtained with the application of seaweed extracts. However, the wide range of physiological responses obtained with application of seaweed extracts implies that more than one group of PGR may possibly be involved (Crouch & van Staden, 1993).

There are numerous commercial seaweed extracts available worldwide. All these extracts are made from brown algae, probably mainly owing to their size and availability rather than their chemical composition (Mooney & van Staden, 1986). Very few seaweed extracts have been extensively investigated to identify the PGRs present in them. The aims of this work were to determine if cytokinin- and auxin-like activity is commonly found in all seaweed extracts and to ascertain the relative levels of activity they may contain.

Materials and methods

A number of commercially used seaweed extracts were obtained (Table 1) and stored at 10 °C.

To determine cytokinin-like activity, the various seaweed extracts (100 ml) were dried down *in vacuo* and resuspended in 100 ml 80% ethanol. These extracts were left overnight at 10 °C and then filtered through Whatman No. 1 filter paper. Free cytokinins were extracted by cation exchange resin chromatography (30 g Dowex 50W-X8). The pH of each seaweed extract was adjusted to 2.5 and the sample passed slow-

Table 1. Details of the seaweed extracts used in this study

Product	Seaweed used in manufacture	pH	Method of preparation
Kelpak	*Ecklonia maxima* (Osbeck) Papenf.	4.5	Cell burst
Marinure	*Ascophyllum nodosum* (L.) Le Jolis	5.2	Al.C.S.D.[1]
Maxicrop	*Ascophyllum nodosum*	7.2	Al.C.S.D
Redicrop	–	7.8	–
Seamac	*Ascophyllum nodosum*	4.8	Al.C.S.D.
SM3	Laminariaceae and Fucaceae species	4.5	Aqueous extract

[1] Al.C.S.D. = Alkaline Caustic Soda Dehydration Process.

ly (20 ml h^{-1}) through the resin. Following a wash with 100 ml 80% ethanol, the cytokinins were released from the resin by 100 ml 5N ammonium hydroxide. This fraction was collected, dried down *in vacuo* and the residue resuspended in 3 ml 80% ethanol. Extracts were applied as 1 cm strips onto Whatman No. 1 chromatography paper and separated by descending chromatography using *iso*-propanol: 25% ammonium hydroxide: water (10:1:1 v/v). When the solvent front was approximately 30 cm from the origin, the chromatograms were removed and dried at 50 °C for 24 h. The chromatograms were divided into 10 equal R_f zones and each analysed for cytokinin-like activity using the soybean callus bioassay (Miller, 1965).

Three pieces of 4-week old soybean callus (var. Acme) of approximately 10 mg were placed on the basal medium and incubated at 25 °C with continuous low light (0.4 μmol m^{-2} s^{-1}) for 28 days after which the callus pieces were weighed. Standards of kinetin (0, 1, 10 and 50 $\mu g\ l^{-1}$) were included with the bioassay.

To determine auxin-like activity, dilution series of the various seaweed extracts were made at the concentrations; 100%, 50%, 20%, 10%, 2%, 1.5%, 1%, 0.2% and 0.1%. Distilled water served as a control. Indole-butyric acid (IBA) over a concentration range of 10^{-7}–10^{-4} M was used as the standard.

The mung bean rooting bioassay as described by Hess (1964) was used to test for auxin-like activity. *Vigno mungo* L. seeds were surface sterilized in 3.5% sodium hypochlorite for 20 min, rinsed thoroughly and then soaked in tap water for 6 h. The seeds were planted in moist vermiculite and germinated at 26 °C in a growth cabinet. After 10 days, uniform hypocotyl cuttings (12 cm) with two primary leaves but with cotyledons removed, were prepared. The cuttings were immediately transferred to vials containing 20 ml of the respective test solutions. Five cuttings were placed in each vial and with two vials per treatment. The vials were placed in a growth cabinet at 26 °C for 8 h. After this pulse treatment, the cuttings were rinsed with tap water and transferred to clean vials containing only water and left in a growth cabinet at 24 °C in low light (9 μmol m^{-2} s^{-1}) with a 16 h light: 8 h dark cycle. The numbers of roots formed after 8 days were recorded.

Results

Cytokinin-like activity was detected in all six of the seaweed extracts tested (Figure 1). The extract with the highest activity, Seamac, yielded the most callus growth at R_f 0.9. This co-chromatographed with *iso*-pentenyladenine (iP) and *iso*-pentenyladenosine (iPA). All the other extracts tested showed callus growth at R_f 0.7–0.8. This co-chromatographed with zeatin (Z) and zeatin riboside (ZR).

All the seaweed extracts tested caused an increase in the number of roots produced on the mung bean cuttings (Figure 2). The optimum dilution for root initiation varied from 1% for Maxicrop to 50% for Marinure. Higher concentrations of the seaweed extracts caused a decrease in rooting. Kelpak produced the highest root count of all the seaweed extracts with the optimum dilution being 20%. This activity was comparable to IBA at 10^{-5}–10^{-4} M.

Discussion

The results indicate that both cytokinin- and auxin-like compounds occur in seaweed extracts. The method of preparation (Table 1) does not appear to significantly alter the amount of biological activity observed for each seaweed extract in the two bioassays. The majority of the commercial seaweed extracts available are produced by an Alkaline Caustic Soda and Dehydra-

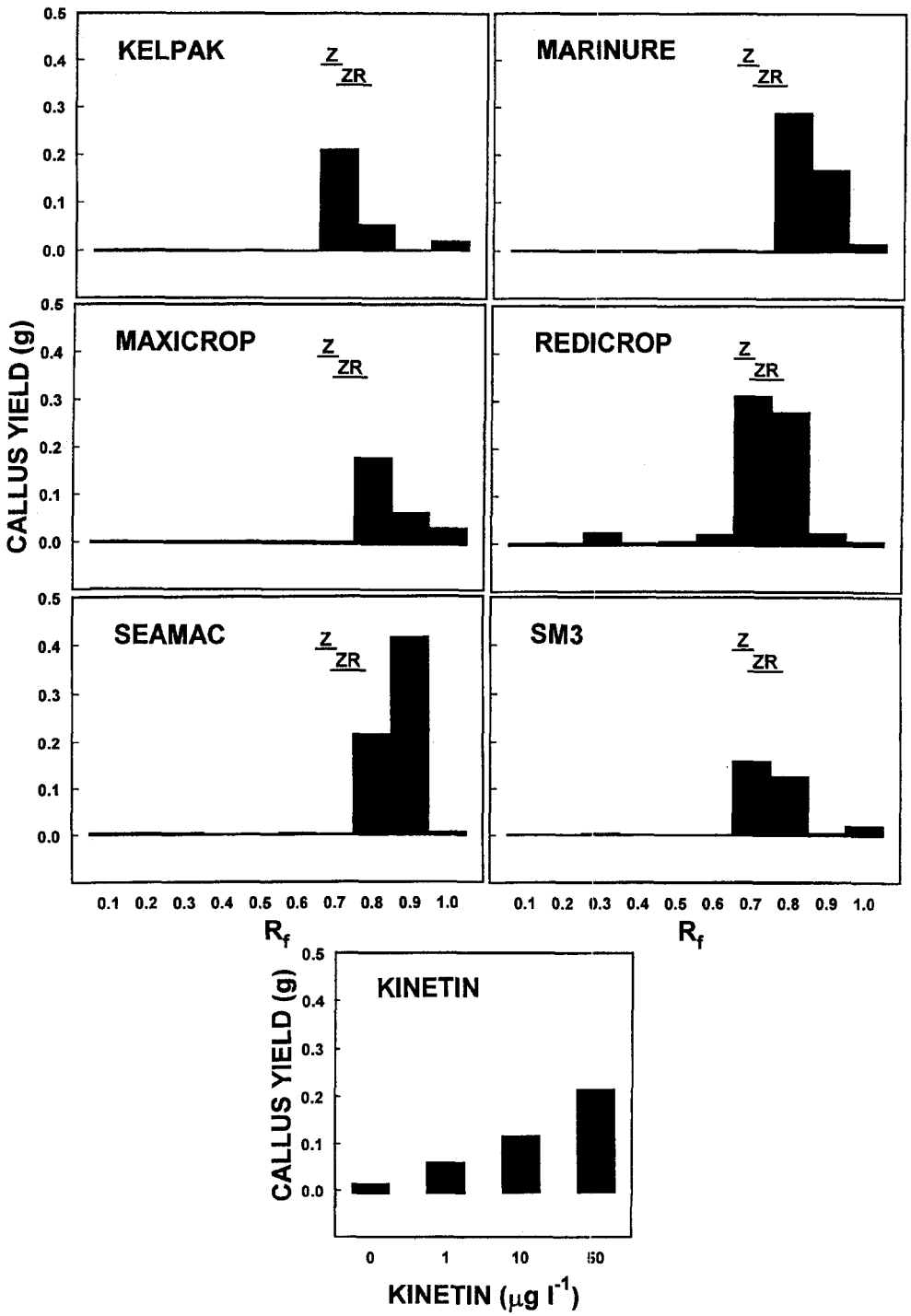

Figure 1. Cytokinin-like activity detected in some commercial seaweed extracts using the soybean callus bioassay. The extract (100 ml) was purified using cation exchange resin and paper chromatography. Z = zeatin and ZR = zeatin riboside.

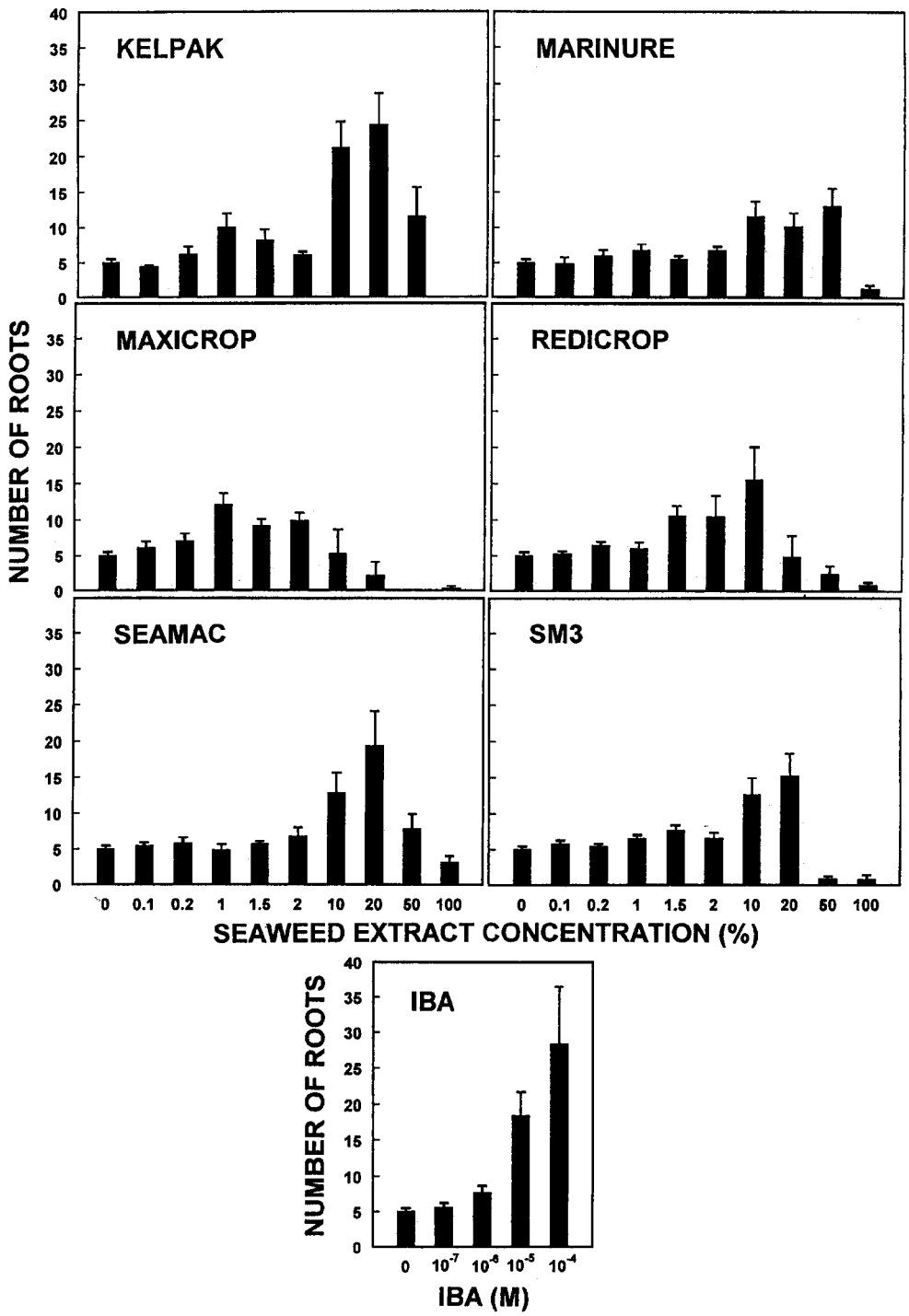

Figure 2. Auxin-like activity detected in some commercial seaweed extracts using the mung bean bioassay.

tion process (Table 1). The cell walls of the seaweed are weakened by alkaline caustic soda which causes the cell contents to be released into a liquid medium. This is then filtered and dehydrated into a water soluble powder. Kelpak is the only seaweed extract produced by a cell burst process where no chemicals or dehydration steps are used. The freshly cut and washed *Ecklonia maxima* stipes are passed through a series of cutters which progressively diminish the particle size. These particles are then subjected to high pressure which induces a degree of potential energy. When the particles are passed at high velocity through a low pressure area, the energy released causes the cell walls to expand, exceeding their elastic limit and thereby rupturing and releasing the cell contents.

There are numerous examples in the literature of cytokinins and auxins being identified in algae. One example is *Ascophyllum nodosum*, one of the most commonly used brown algae in seaweed extracts (Table 1). Kingman & Moore (1982) identified the purine adenine, the auxin indole-3-acetic acid (IAA) and abscisic acid (ABA) in crude extracts of *Ascophyllum nodosum*. However, there have been relatively few studies concerned with the identification of PGRs in commercial seaweed extracts. Most of what has been done concentrated on cytokinins. Brain et al. (1973) were the first to detect cytokinin-like activity in the seaweed extract SM3.

Cytokinins have been positively identified in Seasol, a seaweed extract made from the Tasmanian kelp *Durvillea potatorum* (Labill.) Aresch. The cytokinins identified include *trans*-zeatin (tZ), *trans*-zeatin riboside (tZR) and their dihyro derivatives – dihydrozeatin (DHZ) and dihydrozeatin riboside (DHZR) as well as iP and its riboside iPA. This was done using GC-MS and the soybean callus bioassay. The highest concentration of cytokinin was DHZR at 35.6 μg l^{-1} followed by iP 15.9 μg l^{-1}. The cytokinin with the lowest concentration was tZ at 0.7 μg l^{-1}. The total cytokinin concentration in Seasol was 0.115 mg l^{-1} (Tay et al., 1985). Zeatin-O-glucoside (ZOG) and its dihyro derivative, and dihyrozeatin riboside-O-glucoside (DHZROG) were also identified later (Tay et al., 1987). Sanderson & Jameson (1986) unequivocally identified, by GC-MS, the cytokinins Z, DHZ, iP and iPA in Maxicrop and also detected cytokinin glucosides using the tobacco callus bioassay. They estimated from their bioassay results that there was in the order of 5.4 μg kinetin equivalent activity per litre Maxicrop. Featonby-Smith & van Staden (1983) estimated the total cytokinin activity detected in Kelpak to be 516 ng kinetin equivalent 20 g^{-1} extract. The active compounds co-chromatographed with Z, ZR and ZG.

Sanderson et al. (1987) positively identified auxin (IAA) in Maxicrop using GC-MS and with the aid of internal markers, estimated the concentration to be 6.63 μg g^{-1} DW (powder form of Maxicrop). Using GC-MS, Crouch & van Staden (1992) identified IAA, indole-3-carboxylic acid (ICA), N'N-dimethyltryptamine, indole-3-aldehyde (IAId) and *iso*-indole,1,3-dione (N-hydroxyethylphthalimide) in Kelpak.

It is accepted that when bioassays are used to indicate biological activity, they must be used with extreme caution when estimating the concentration of an active compound. Structurally different compounds respond differently in various bioassays, some bioassays being less sensitive to different compounds. Also, the various compounds show different levels of activity e.g. zeatin is the most active naturally occurring cytokinin in higher plants (Letham, 1978). Thus, depending on the bioassay used and the compounds present in the extracts, the amount of activity detected can vary.

Another consideration when comparing the biological activity associated with seaweed extracts, is the time at which the plant material was harvested. It is known that cytokinin concentrations vary seasonally in the brown algae *Ecklonia maxima* (Featonby-Smith & van Staden, 1984), *Sargassum heterophyllum* (Turn.) J. Ag. (Mooney & van Staden, 1984) and *Macrocystis pyrifera* (L.) C. Ag. (de Nys et al., 1990). In all three of the above mentioned studies, the authors found an accumulation of cytokinins in actively growing tissues which correlated to seasonal growth patterns. Ideally, when comparing the activity in the seaweed extracts, they should all have been harvested in the same growing phase or season.

The results of this study indicate that the levels of cytokinin- and auxin-like activity are similar for all the seaweed extracts tested.

Acknowledgments

The Foundation for Research and Development, Pretoria and the University Research Fund are thanked for financial support.

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