Chapter 14
Production of Plant Hormones from Algae and Its Relation to Plant Growth

Sanaa M. Shanab and Emad A. Shalaby

Contents

S. M. Shanab
Botany and Microbiology Department, Faculty of Science, Cairo University, Giza, Egypt

E. A. Shalaby (✉)
Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt

1 Introduction

Plant growth regulators (such as auxins, cytokinins, gibberellin, abscisic acid, and ethylene) play an important role in mediating growth of different plant species at very low concentrations as well as signaling environmental alterations or changes, initiating stress responses (biotic and abiotic) and indicator molecules in the regulation of almost all phases of plant growth and development (maturation) from embryogenesis to senescence (Li et al. 2010).
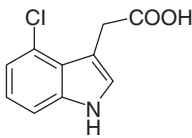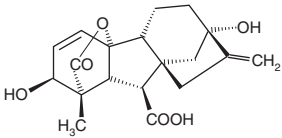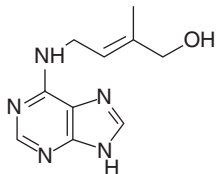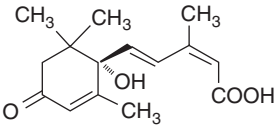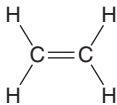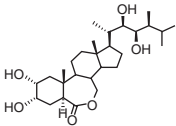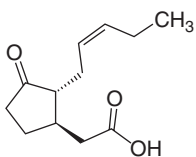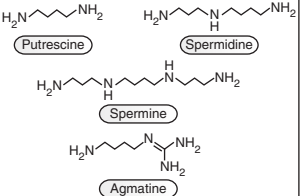
Seaweed and microalgae extracts are used as growth stimulants or growth regulators in cultivation of agricultural species due to its content from the plant growth regulator (hormones) concentrations (Stirk and Van Staden 2006).

In various algal species, phytohormones were recorded in significant amount when compared with their amount in plants, and the various biological activities of algal hormones corresponded to the functions of hormones in higher plant (Tarakhovskaya et al. 2007).

El Shoubaky and Salem (2009) investigated green macroalgae (*Ulva lactuca* and *Enteromorpha clathrate*) as biofertilizers due to their high concentrations of inorganic nutrients in addition to organic compounds and plant growth hormones. Phytohormones of microalgae and macroalgae are exogenous growth regulators, affecting the tolerance ability to different factors of various (abiotic and biotic) stress conditions (Romanenko et al. 2015).

Hormonal level may undergo changes when the alga is exposed to an alteration in natural environmental or laboratory conditions such as light (quantity, quality, and duration), temperature, salinity, etc. Moreover, Nimura and Mizuta (2002) reported that the endogenous abscisic acid (ABA) content of laminaria sporophyte increased as a result of its transition from the vegetative growth stage to the reproductive state. Also, in some microalgae, ABA was increased under salt stress or lowered moisture content. Polyamines and betaines are important active signal factors required for different processes in plant and algal development and participate in biotic and abiotic stress responses as illustrated by Kusano et al. (2008). These factors were recorded in different stressed microalgal and seaweed species (Mackinnon et al. 2010; Gebser and Pohnert 2013).

In the following sections, we will summarize the major plant hormones and its analogues present in algal species and its physiological functions and methods of extractions and determinations and if any abiotic stress factors can affect the accumulation of these regulators inside the algal species.

Auxin	**Gibberellin**
Cytokinins	**Abscisic acid**
Ethylene	**Brassinosteroids**
Jasmonic acid	**Polyamines**

The chemical structure of major phytohormones and other regulators produced by algae

2   Algal Hormones

2.1   *Auxins*

It is interesting to know that not only the hormonal substances produced by the highly evolved terrestrial plants are already produced by the lower primitive thallophytic algae, but they also function similarly. Regarding auxins, there have been numerous investigations that dealt with auxin production (especially IAA) in many algal species (microalgae, macroalgae, and cyanobacteria).

Starting with Du Buy and Olson (1937) who reported the presence of auxin in the tissue of *Fucus vesiculosus*, occasionally, other scientists recorded the presence of auxin in *Bryopsis muscosa*. Also, indole acetic acid (IAA) were found in the brown seaweeds *Fucus*, *Macrocystis*, and *Desmarestia* sp. Few years later (Skibola 2004; Tarakhovskaya et al. 2007; Li et al. 2007), a growth substance similar to IAA was

found in *Laminaria agardhii*. These previous investigations proved their findings by the use of bioassay of Avena coleoptile curvature.

This was followed by a lot of investigations which dealt with the presence of auxinic substance in many algal species as well as the isolation and identification of indolic substance using different analytical methods. The previously recorded studies confirmed the production of auxins in various algal species belonging to different divisions, but they also proved that the hormonal function and its catabolism followed the same pathway as that in angiosperms (Sitnik et al. 2003; Stirk et al. 2009). Researches continued in this field till now, recording the presence of one or more plant hormones in different algal species, identifying its (or their) chemical structure by chromatographic analysis, and confirming its hormonal properties by specific plant bioassays (Table 14.1 and Figs. 14.1, 14.2, and 14.3). Auxins in algal thalli varied from season to season, and developmental stage and highest concentration were recorded especially in summer season and in vegetative tissues (EL Shoubaky and Salem 2016; Mori et al. 2017).

The effect of different culture conditions especially a biotic stress (such as concentration of L-tryptophan, acidity degree, and light conditions) on the synthesis of indole by *Spirulina* sp. was reported by Ahmed et al. (2010). It was found that the formation of IAA in *Spirulina* sp. was organized by 1.5 μg/mL L-tryptophan concentration. Moreover, the height amount of IAA was found at pH 6 in light-dark cycle 8:16 h. However, in the dark, auxin synthesis was not observed.

IAA is the naturally occurring growth regulator in the kingdom of plant. It is present in very low concentration (0.5–15 μg/kg) and is in equilibrium with bound forms (such as glucose, ester, aldehyde, and more complex forms as glucobrassicin).

IAA is synthesized in different plants from the tryptophan (amino acid) by different biosynthetic routes.

2.1.1 Physiological Properties of Auxins

1. Initiation of root formation.
2. Apical dominance.
3. Tropisms.
4. Differentiation of phloem elements.
5. Induction of elongation.

2.1.2 Separation and Detection

Auxins can be separated by paper chromatography (PC) using the mobile phase/isopropanol/ammonium hydroxide/water (8:1:1).

It can be separated by thin layer chromatography (TLC F_{254}) using the mobile phase/chloroform/ethyl acetate/formic acid (5:4:1); standard indoles are used for comparison.

Table 14.1 Seaweed species producing auxins

Algal species	Division
Fucus vesiculosus	Phaeophyta
Macrocystis sp.	
Desmarestia sp.	
Fucus sp.	
Ascophyllum sp.	
Ascophyllum nodosum	
Laminaria agardhii	
Laminaria sp.	
Undaria pinnatifida	
Pylaiella littoralis	
Fucus vesiculosus	
Ecklonia maxima	
Macrocystis pyrifera	
Dictyota humifusa	
Sargassum heterophyllum	
Laminaria japonica	
Valonia utricularis	Chlorophyta
Valonia macrophysa	
Bryopsis muscosa	
Acetabularia sp.	
Cladophora sp.	
Ulva pertusa	
Enteromorpha compressa	
Caulerpa paspaloides	
Enteromorpha prolifera	
Ulva fasciata	
Ulva lactuca	
Ulva rigida	
Gelidium amansii	Rhodophyta
Eisenia bicyclis	
Ceramium rubrum	
Botryocladia sp.	
Porphyra sp.	
Pyropia yezoensis	
Bangia fuscopurpurea	
Sarconema filiforme	
Nemalion multifidum	
Furcellaria fastigiata	

To detect the separated spots, they can be sprayed with the coloring reagent DMAC (0.1 g of *p*-dimethylaminocinnamaldehyde in 10 mL concentrated HCl, then diluting to 200 mL with acetone). In case of using PC for separation, the PC paper will be dipped in the reagent, dried, and then heated at 65 °C for 2.5 min.
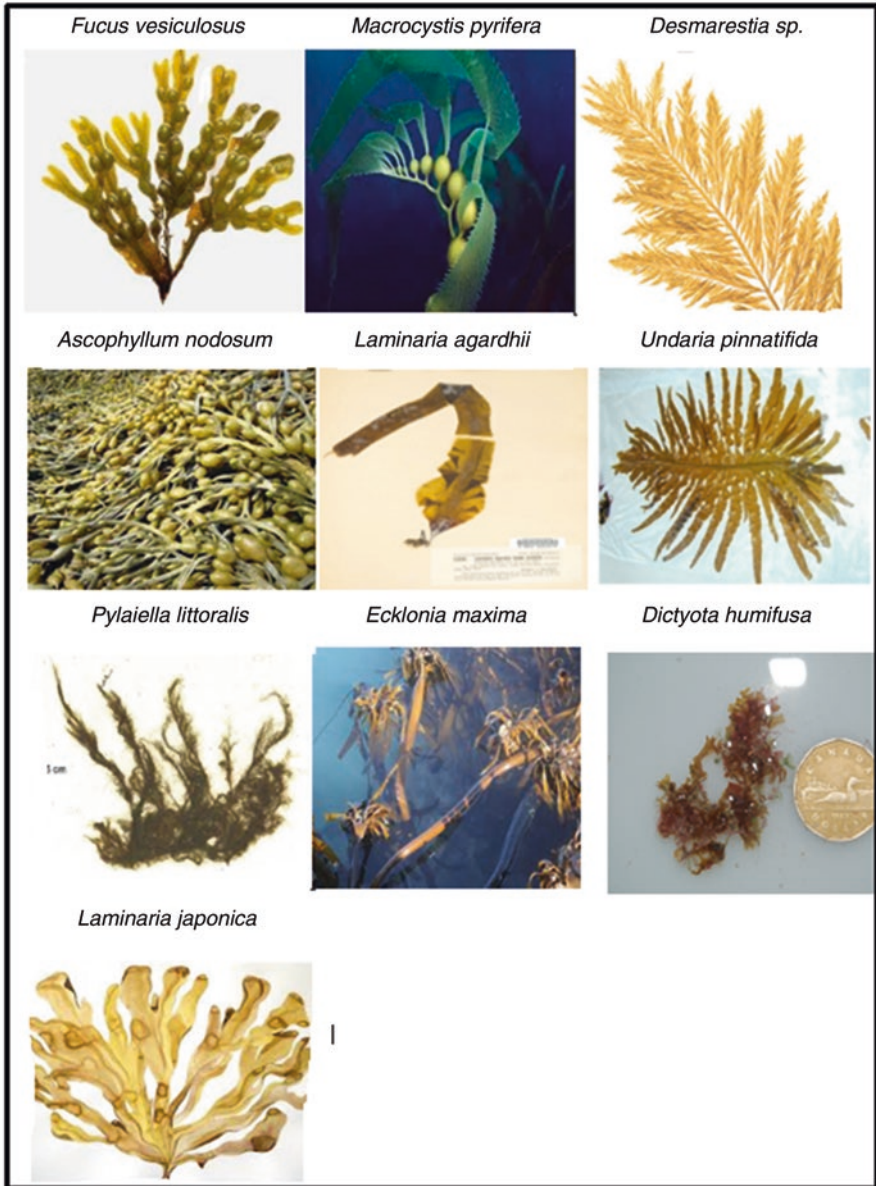
Fig. 14.1 Some Phaeophyta species producing auxins
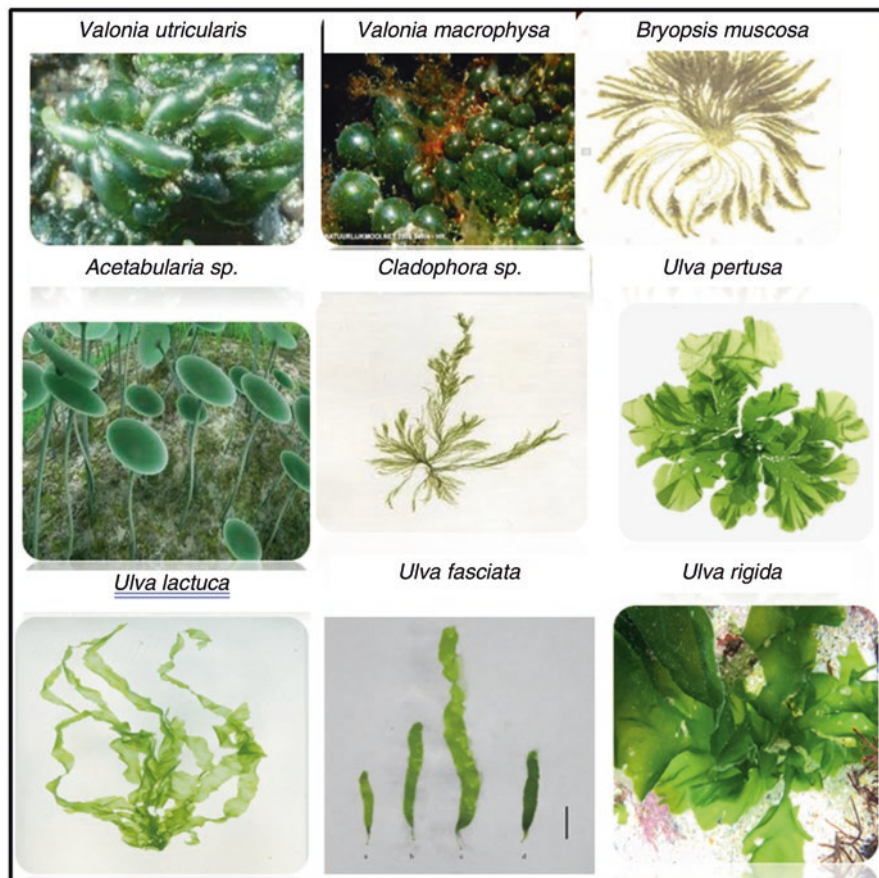
Fig. 14.2 Some Chlorophyta species producing auxins

Another coloring reagent can be used in case of PC which is called Salkowski reagent (0.001 M ferric chloride in 5% perchloric acid), giving pink spots with $FeCl_3$/perchloric acid/ethanol reagent. Using TLC for separation of the indolic extract, the spray reagent is used as 0.25% of DMCA in ethanol/conc. HCl (1:1) and the color will develop over night at room temperature (purple spot).

2.1.3 Identification and Determination

For identification of indoles, spectral measurements in methanol must be at wavelength 220–320 nm. Indole acetic acid has fluorescence peak of 365 nm and activity peak of 285 nm. Identification of auxins are performed by LC/MS, GLC/MS, HPLC/MS, and GC/MS, which identify the indolic compound(s) (compared with
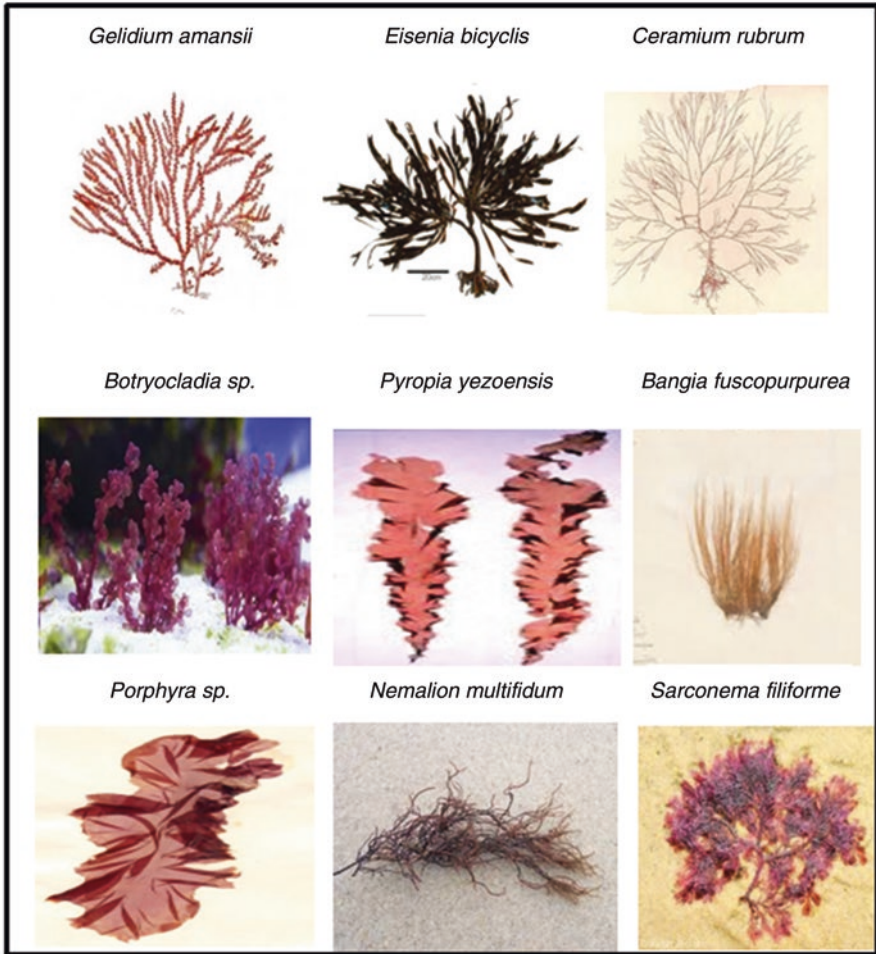
Fig. 14.3 Some Rhodophyta species producing auxins

either standard or not) at its specific retention time, and recording its chemical structure and formula.

2.1.4 Measurement of Its Characteristic Biological Activity

1. Using suitable plant bioassays (for each hormone compared to the control of synthetic standard).
2. Avena coleoptile curvature test.
3. Elongation of wheat (or barley) coleoptile sections.
4. Induction of rooting in cutting stem of mung bean.

2.2   Cytokinins

They are group of plant growth regulators consist of purines substituted in the six position. The first naturally occurring cytokinin was zeatin (from *Zea mays*) which was commonly found as riboside forms. Cytokinin was found to initiate all division in plant tissue culture during growth. It interferes with auxins in many developmental stages controlled by the balance on the ratios of cytokinin to indoles which may influence shoot and root differentiation and growth of lateral buds or remain at the undifferentiated callus stage. Cytokinins are synthesizing from adenosine-5-monophosphate producing iso-pentenyladenine (ip) which was believed to be the precursor of all other naturally occurring cytokinins. Different cytokinin-like substances were reported in different algal species belonging to various algal groups, as zeatin, dihydrozeatin, iso-pentenyladenine (ip), *N*6-methylaminopurine, and *N*6,8,8-dimethyl-allylaminopurine.

In Phaeophyta, Stirk et al. (2003) recorded cytokinin-like activity in *Fucus serratus*, *Ascophyllum nodosum*, *Ecklonia maxima*, *Laminaria saccharina*, *Fucus vesiculosus*, *Dictyota* sp., and *Sargassum heterophyllum* (coincide with the release of gametes), while Stirk and Van staden (1997) reported the cytokinin activity in the green seaweeds: *Ulva* sp., *Cladophora contexta*, *Codium capitatum*, *C. extricatum*, *Halimeda cuneata*, *Caulerpa racemosa*, and *Valonia macrophysa*.

Detection of cytokinin-like activity in the red seaweeds (Rhodophyta) was achieved by Yokoya et al. (2010) and Mori et al. (2017), who reported that this activity was demonstrated in *Galaxaura diesingiana*, *Gelidium amansii*, *Amphiroa bowbankii*, *A. ephedraea*, *Arthrocardia* sp., *Cheilosiphorum sagiltatum*, *Jania crassa, Plocamium corallorhiza*, *Hypnea rosea*, *H. spicifera*, *Spyridia hypnoides*, *Pyropia yezoensis*, and *Bangia fuscopurpurea*. In various algal groups (Stirk et al. 2009; Mori et al. 2017), aromatic cytokinins (topolins) were recorded as shown in Table 14.2 and Figs. 14.4, 14.5, and 14.6.

Regarding the response of algal cells to abiotic stress condition and its relation to phytohormone accumulation, Maršálek et al. (1992) found that during cultivation of microalgae species, the concentration of ABA in mother algal cultures was three times folded when compared with new culture. An increase in ABA level was observed during the first 24 h of microalgae cultivation in the absence of light condition and decreased in the following 24 h; at the same time, the amount of ABA was decreased gradually in light and dark environment conditions (14:10) (Stirk et al. 2014). Also, ABA softened the effect of various oxidative stress conditions, with positive relation to the activity of AO enzymes such as glutathione *S*-transferase, peroxidase, and catalase (Yoshida et al. 2003).

2.2.1 Separation and Detection

Separate the ammoniacal fraction by descending paper chromatography using propanol/ammonia/water (10:1:1) as described by Stirk and Van staden (1997) and using standard cytokinin (as kinetin) for comparison. t-Butanol/conc. NH_4OH/H_2O (3:1:1) or n-butanol/acetic acid/water (4:1:1) can be used as mobile systems. The separated cytokinin appears as dark spots in short UV light. Using TLC of alumina G, separation of cytokinin (as zeatin) can be performed using butanone saturated with water or EA saturated with H_2O or by chloroform/EthOH (9:1). Detection done using Dische reagent (spraying with 0.5 g cysteine hydrochloride in $3MH_2SO_4$ giving a pink color after 20 min).

Table 14.2 Seaweed species producing cytokinins

Algal species	Division
Fucus vesiculosus	Phaeophyta
Desmarestia sp.	
Undaria pinnatifida	
Bifurcaria brassicaeformis	
Ascophyllum nodosum	
Sargassum muticum	
Laminaria japonica	
L. pallida	
Macrocystis pyrifera	
Ecklonia maxima	
Laminaria saccharina	
Dictyota sp.	
Dictyota humifusa	
Sargassum heterophyllum	
Macrocystis angustifolia	
Splachnidium rugosum	
Ulva sp.	Chlorophyta
Ulva fasciata	
Cladophora coelothrix	
Codium capitatum	
C. extricatum	
Halimeda cuneata	
Caulerpa racemosa	
Caulerpa filiformis	
Valonia macrophysa	

(continued)

Table 14.2 (continued)

Algal species	Division
Amphiroa bowbankii	Rhodophyta
A. ephedraea	
Arthrocardia sp.	
Plocamium corallorhiza	
Cheilosporum sp.	
Hypnea spicifera	
Galaxaura diesingiana	
Gelidium amansii	
Cheilosporum sagittatum	
Jania crassa	
Hypnea rosea	
Griffithsia pacifica	
Pyropia yezoensis	
Bangia fuscopurpurea	
Aeodes orbitosa	
Gigartina clathrata	
Gigartina polycarpa	
Sarcothalia scutellata	
Hymenena venosa	
Nothogenia erinacea	
Plocamium corallorhiza	
Carradoeriella virgata	
Porphyra capensis	
Sarcothalia stiriata	
Suhria vittata	
Amphiroa bowerbankii	
Arthrocardia sp.	
Cheilosporum sp.	
Jania sp.	

2.2.2   Identification and Determination

Occur by using HPLC.

2.2.3   Bioassay/Biological Activity

The ability of cytokinin to promote growth of secondary phloem of carrot. The effect of cytokinin on barley germination.

Fig. 14.4 Some Phaeophyta species producing cytokinins

The cytokinin-like activity promoting cell division can be assayed by soybean callus culture.

2.2.4 Physiological Properties of cytokinins

1. Shoot and Root differentiation in tissue culture.
2. Growth of lateral buds and leaf expansion.
3. Chloroplast development.
4. Leaf senescence.
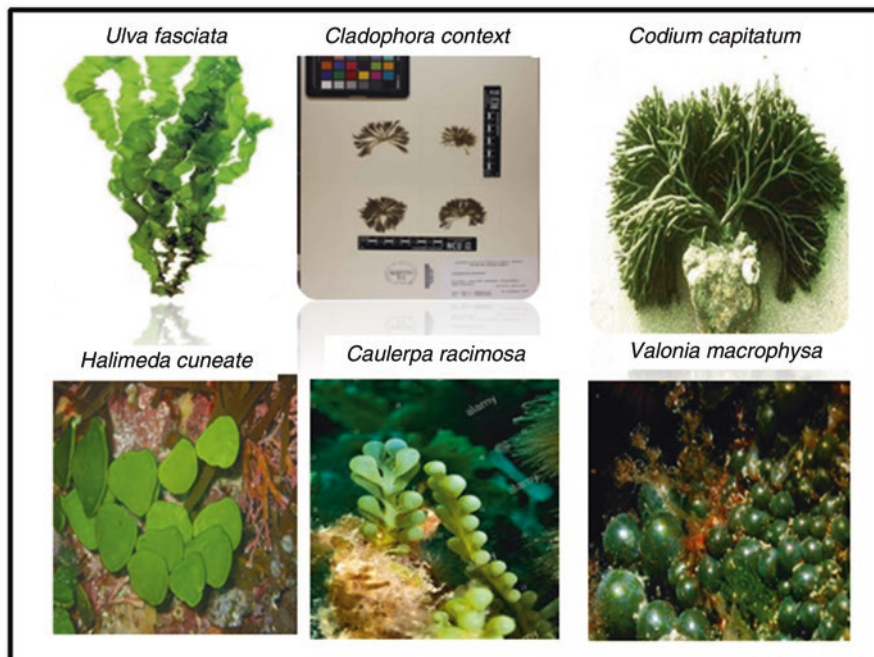5. Morphogenesis in cultured tissues.

Fig. 14.5 Some Chlorophyta species producing cytokinins

2.3 Gibberellins (GAs)

It is clear from literatures that gibberellin-like substances are synthesized by different types of macroalgae. Bently (1960) suggested the presence of GA_3 like substances in microalgae and macroalgae. She revealed that there are two unknown components in acidic extracts of phytoplankton which have some growth stimulatory characteristics. Meanwhile, Stirk et al. (2013a, b) extracted gibberellin-like substance from *Fucus vesiculosus*, purified it by PC and identified one or an analogue of GA_1, GA_3, and GA_6 (~10 μg/kg F.wt) (R_f = 0.3–0.4). Furthermore, other data found gibberellin-like activity in *Fucus spiralis* (GA_1 or GA_3).

Detection of gibberellins in other brown seaweeds was recorded by many investigations (EL shoubaky and Salem 2016). Moreover, these scientists recorded gibberellin in the red seaweeds *Hypnea musciformis*, *Gracilaria corticata*, and *Porphyra leucostricta*. Table 14.3 and Figs. 14.7, 14.8, and 14.9 recorded some algal species producing gibberellins. Researches continued till now searching for plant hormones in seaweed species and applying recent techniques for their extraction, separation, identification, and determination (Stirk et al. 2013a, b). Gibberellins are group of hormones (belongs to diterpenoids) which stimulate plant growth and are widespread in plants and algae.
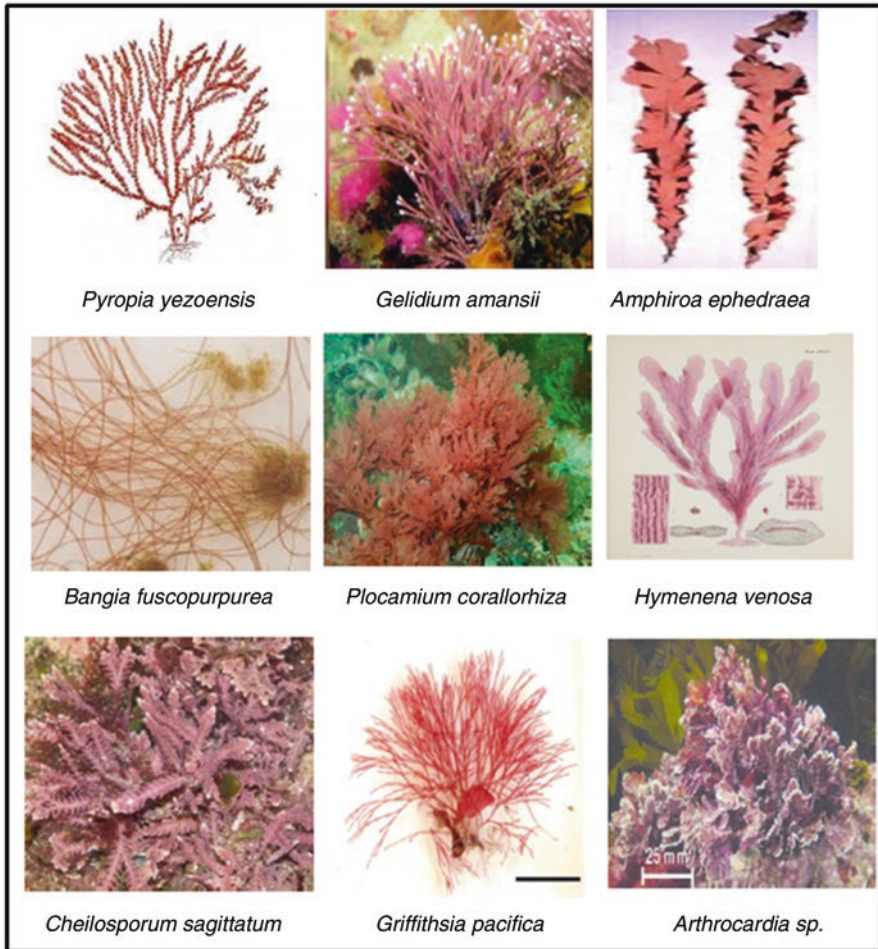
Fig. 14.6 Some Rhodophyta species producing cytokinins

In fact, more than 40 compounds of gibberellin structure have been recognized till now. The most familiar is gibberellic acid (GA_3).

2.3.1 Physiological Properties of Gibberellins

1. It promotes seed germination and organ differentiation.
2. It stimulates stem elongation and shoot growth.
3. It interferes with leaf expansion, development, and fruit maturity (Yamaguchi 2008; Sun 2010). It has positive effect with IAA to differentiation of cell and elongation of root but has negative effect with abscisic acid on growth and ger-

Table 14.3 Seaweed species
producing gibberellins (GAs)

Algal species	Division
Cystoseira sticta	Phaeophyta
Fucus vesiculosus	
Fucus spiralis	
Sargassum plagiophyllum	
Ecklonia radiata	
Ascophyllum nodosum	
Enteromorpha flexuosa	Chlorophyta
Oedogonium cardiacum	
Caulerpa prolifera	
Codium fragile	
Enteromorpha prolifera	
Ulva lactuca	
Ulva rigida	
Gracilaria corticata	Rhodophyta
Porphyra leucostricta	
Hypnea musciformis	
Sarconema filiforme	
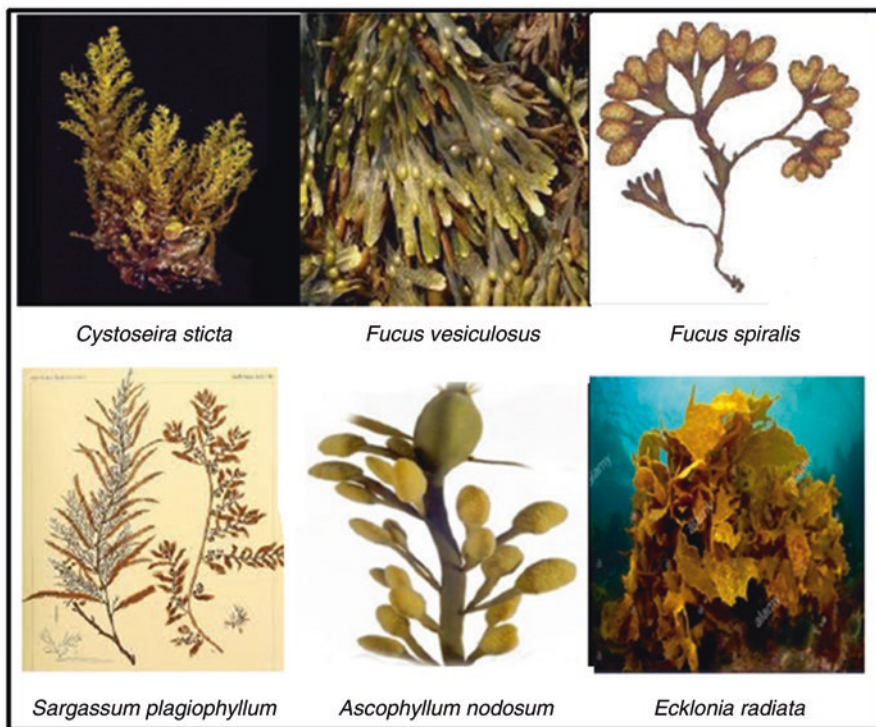
Fig. 14.7   Some Phaeophyta species producing gibberellins

Enteromorpha flexusa   Oedogonium cardiacum   Caulerpa prolifera

Codium fragile   Enteromorpha prolifera   Ulva rigida

Ulva lactuca
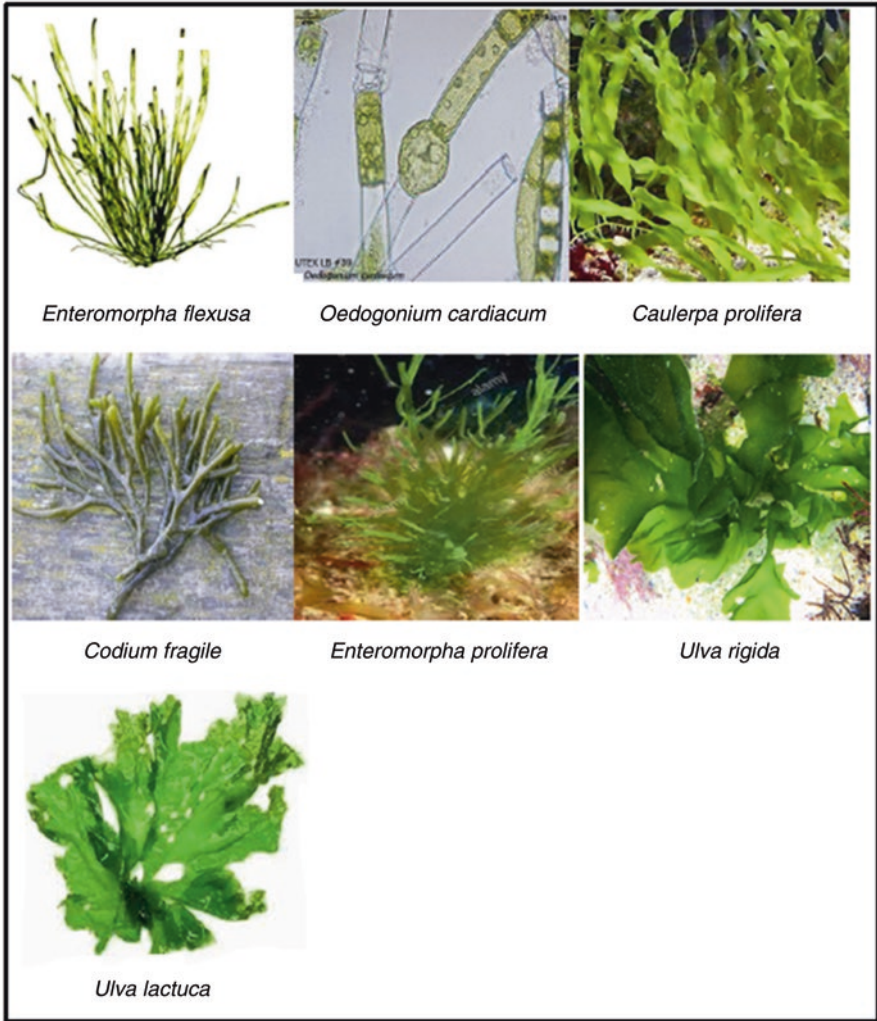
Fig. 14.8 Some Chlorophyta species producing gibberellins

mination. Negative and positive effects depend on environmental conditions and factors with stress-related ethylene and negative effects with cytokinin concentration (Weiss and Ori 2007; Yamaguchi 2008). Gibberellins were synthesized from glyceraldehyde 3-phosphate in young shoot tissues and developing seeds.
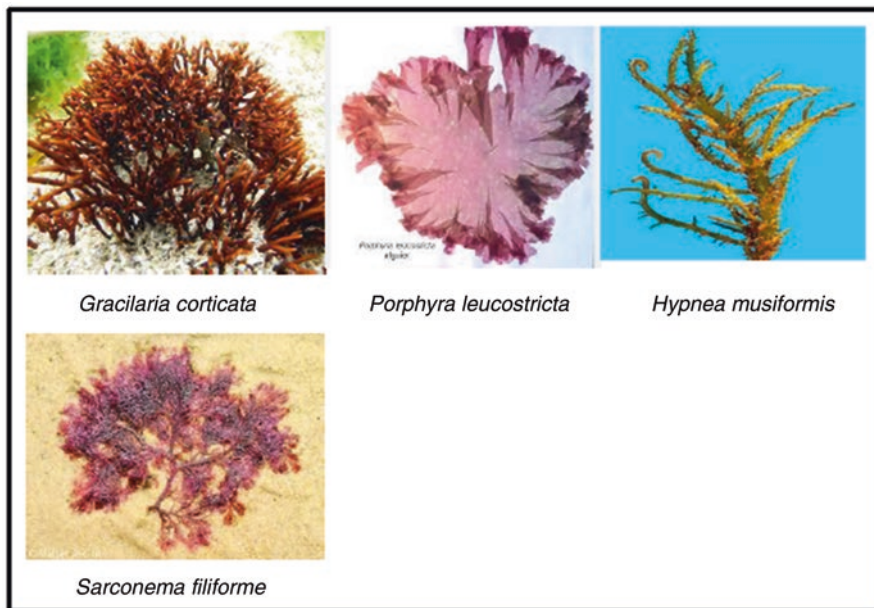
Fig. 14.9 Some Rhodophyta species producing gibberellins

2.3.2   Separation and Detection

Gibberellins contain more than 40 chemically closely related compounds which are difficult to separate and distinguish. Separation of gibberellins can be performed on column of 5% OV-22, on DMCS-treated chromosorb W.

Gibberellins are separated on silica gel plates with the solvent system benzene/butanol/acetic acid (70:25:5) and benzene/acetic acid/water (50:19:31). Detection was carried out by H_2SO_4/water (7:3) spraying the plate and then exposure at 120 °C; GA_3 appear as spots with yellow-green.

2.3.3   Identification and Determination

The most satisfactory method for gibberellin determination is by GC/MS; using GLC, gibberellins converted first to their methyl ester (by Methylation) or TMS esters (silylation).

2.3.4   Plant Bioassays (Measurement of Gibberellin Biological Activity)

1. Lettuce hypocotyl.
2. α-amylase,
3. Dwarf rice leaf.

2.4   Abscisic Acid (ABA) and Lunularic Acid

It is a sesquiterpenoid growth inhibitor (inhibitor of elongation), but it may significantly stimulate maize root elongation at some concentrations. ABA is present in several plant species in root tips and root caps. White light and stresses (mineral starvation and leaf dehydration) induce high content of ABA in leaves. In the brown algae of genus *Ascophyllum* (*A. nodosum*) and some species of *Laminaria* (Nimura et al. 2002), a hormone was detected which suppressed plant growth in bioassay.

In various algal groups, the growth-inhibiting complex includes lunularic acid and abscisic acid, and other undifferentiated biologically active compounds were recorded.

Lunularic acid is a kind of growth inhibitor which was detected in liverworts. Its structure, activity, and metabolism resemble those of ABA. It suppressed the growth of cut discs from cultured *Laminaria japonica* and induced reproductive tissue formation at the same concentration used in higher plants (10^{-6} to 10^{-4} M). Also, ABA induced the morphogenesis of *Hypnea pluvialis* cells to form cysts. In some microalgae, the endogenous ABA content increased under stress conditions (salinity, light intensity, drought, etc.).

El shoubaky and Salem (2016) recorded ABA in the green seaweeds *Ulva rigida* and *Ulva lactuca* as well as in the red *Sarconema filiforme*, and higher concentration of ABA was recorded in *U. lactuca* where the ABA profile (by GC/MS) contained *cis*-, *trans*-ABA-L-alanine methyl ester, *cis*-, *trans*-ABA-L-valine, and *cis*-, *trans*-ABA-L-alanine. Stirk et al. (2009) detected endogenous ABA in the green *Ulva fasciata* and the brown *Dictyota humifusa*. It was also detected in different seaweed commercial extracts as Kelpak R from *Ecklonia maxima.* Also, red seaweeds were found to produce ABA as in the case of *Bangia fuscopurpurea* and *Pyropia yezoensis* by Mori et al. (2017), as well as red algae of Brazil (Yokoya et al. 2010), as illustrated in Table 14.4 and Figs. 14.10 and 14.11.

2.4.1   Separation, Detection, and Determination

Using paper chromatography for separation of ABA in the algal extract and standard ABA (at conc. 10^{-6} M) for comparison. Detect the isolated spots by UV light of 254 nm as dark absorbed spots. The most widely used techniques for quantification are GC/MS, GLC/MS, and HPLC/MS.

2.4.2   The Biological Activity of ABA Using Specific Bioassays

1. Inhibition of elongation of wheat coleoptile sections (ABA of more than 10^{-8} M).
2. Induction of stomatal closure.

Table 14.4 Seaweed species producing abscisic acid (ABA) and lunularic acid

Algal species	Division
Ascophyllum nodosum	Phaeophyta
Laminaria japonica	
Dictyota humifusa	
Laminaria sp.	
Ascophyllum sp.	
Laminaria digitata	
Ulva rigida	Chlorophyta
Ulva lactuca	
Enteromorpha compressa	
Ulva fasciata	
Sarconema filiforme	Rhodophyta
Bangia fuscopurpurea	
Pyropia yezoensis	

2.5   Ethylene

Plants and macroalgae produce a range of volatile compounds, such as alcohols, alkane, alkenes, esters, etc.

These volatile compounds are produced in response to biotic or abiotic stimuli. These compounds have several biological roles in higher plants such as promotion of seed germination, inhibition of the stem, root elongation, ripening of fruits, senescence of leaves and flowers, and sex determination as reported by Bleecker and Kenode (2000), Klee (2004), Grennan (2008), Holopainen and Gershenzon (2010), and Loreto and Schnitzler (2010). Most of the biosynthetic pathways of volatile compound production depend on *S*-adenosylmethionine compound which may act as a substrate for the enzyme reactions or as a source of methyl group (as in the synthesis of jasmonates, salicylates, and brassinosteroids). Ethylene and dimethyl sulfide are examples of etherial compounds which are produced from the red alga *Gelidium* sp.

The concentrations and types of these compounds were affected by various abiotic stress factors as salinity, light quality, and exogenous ethylene. The period of light and darkness causes the production of amines and methyl alkyl compounds.

Reaction oxygen species (ROS) act as a secondary messenger initiating a signal cascade which stimulate ethylene synthesis (Mackerness 2000). Accumulation of volatile compounds was recorded after the exposure to red light and application of exogenous ethylene.

The level of dimethyl sulfide (DMS) which emitted in all conditions didn't increase after incubation with ethylene (they appear to be not coordinated as reported in the red alga *G. arbuscula*. In *Acetabularia mediterranea*, the rate of algal development decreases with increase of ethylene production.

In *Enteromorpha intestinalis*, reduction of chlorophyll content below that of control occurred on the addition of ethephon which decompose to generate ethylene as

A) Phaeophyta

Ascophyllum nodosum   Laminaria japonica   Dictyota humifusa

Laminaria digitata

B) Chlorophyta

Ulva fasciata   Ulva lactuca   Ulva rigida

Enteromorpha compressa

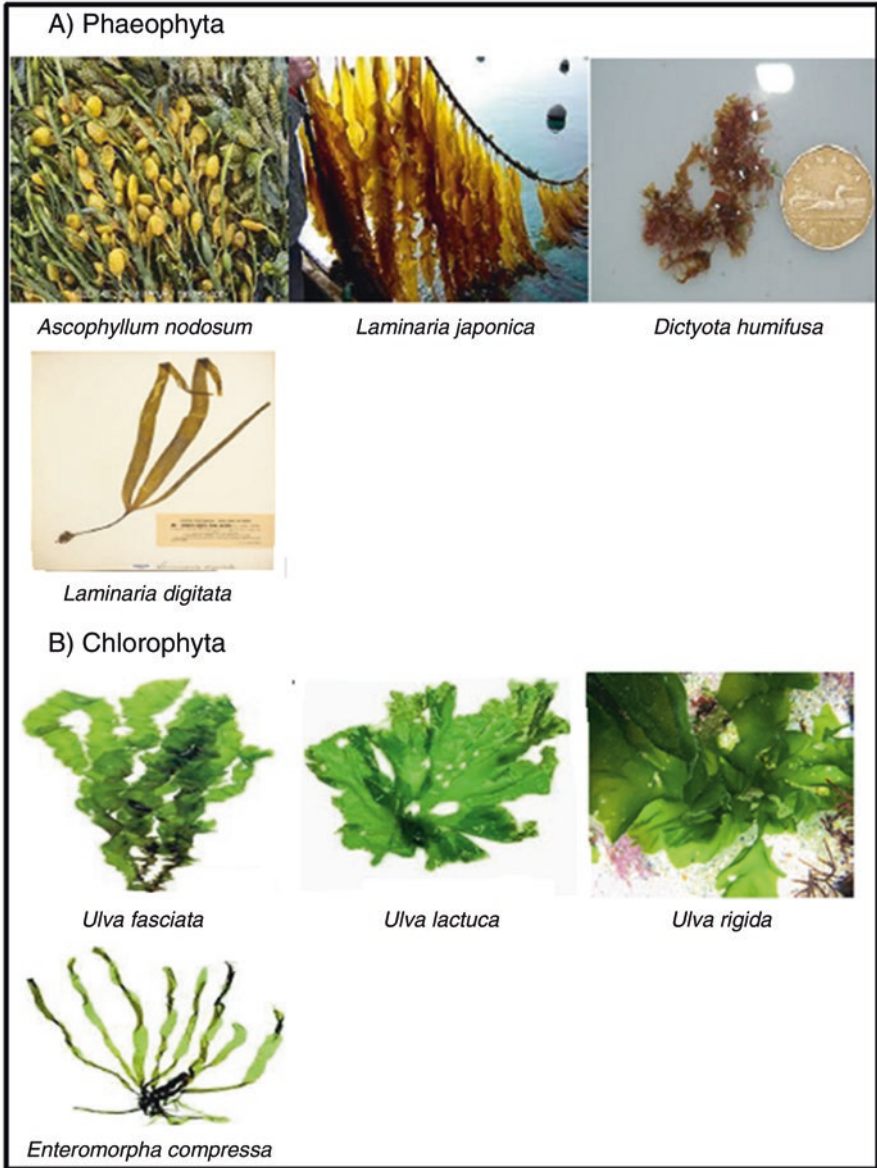**Fig. 14.10** Some (**a**) Phaeophyta and (**b**) Chlorophyta species producing abscisic acid and lunularic acid

reported by Garcia-Jimenez et al. (2013). Moreover, ethylene was involved in growth of the red alga *Pterocladiella capillacea* (Garcia-Jimenez and Robaina 2012).

When the acclimatized *Ulva intestinalis* to low light intensity was transferred to high light condition, ethane level was increased causing an inhibition of chlorophyll
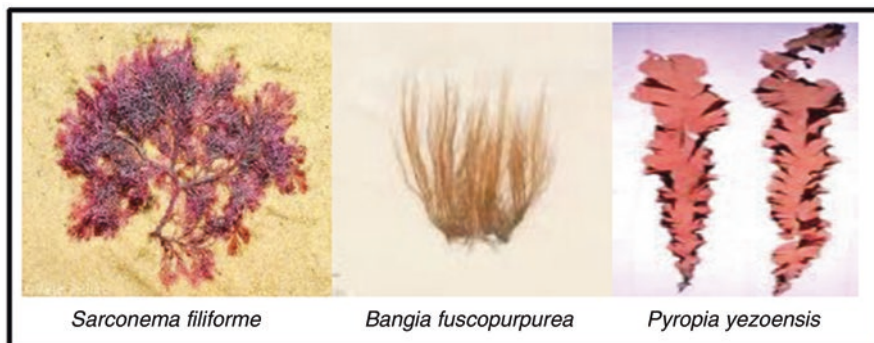
Fig. 14.11 Rhodophyta species producing abscisic acid and lunularic acid

Table 14.5 Seaweed species producing ethylene

Algal species[a]	Division
Ulva intestinalis	Chlorophyta
Acetabularia mediterranea	
Pterocladiella capillacea	Rhodophyta

[a]No Phaeophyta species were recorded

content (by 30%). Table 14.5 and Fig. 14.12a, b recorded some algal species producing ethylene.

2.5.1   Gas Chromatography/MS Analysis of the Released Volatiles (GC/ MS)

Volatile compounds are analyzed using Varian 431GC/210MS with capillary column and He as a carrier gas.

2.5.2   Physiological Properties of Ethylene

Ethylene production increased during leaf abscission, flower senescence, and fruit ripening.

Physiological stresses and wounding induce ethylene biosynthesis.

During storage of fruits, vegetables, and flowers, an effective ethylene absorbent is used ($KMnO_4$, pot. permanganate) to reduce ethylene concentration in the storage area (extending the storage life of the fruits).
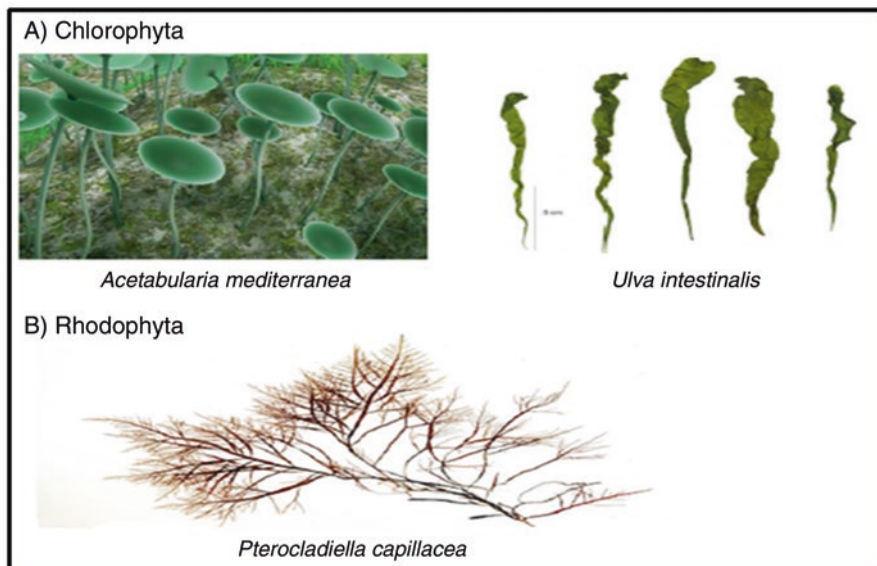
Inhibition of Ethylene Action.

Fig. 14.12 Seaweed species producing ethylene. (**a**) Chlorophyta and (**b**) Rhodophyta

Ethylene effects can be antagonized by silver ion Ag^+ [in the form of $AgNO_3$ or silver thiosulfate $Ag(S_2O_3)_2$]. Also, CO_2 at high concentrations (5–10%) inhibit many ethylene effects.

3 Growth Substances (Growth Regulators)

There are five classical phytohormones detected in angiosperms and in the lower plants as algae. They are auxins, cytokinins, gibberellins, abscisic acid, and ethylene which control different physiological and developmental processes. Their extraction, separation, detection, and determination by various methods are well known. Also, their synthetic pathway(s) and biological functions and bioassays have long been documented. Different chemical compounds were reported to control growth and ameliorate plant (or algal) defensive system against biotic or abiotic stresses. These compounds are termed growth substances or growth regulators; they include brassinosteroids, jasmonic acid, salicylic acid, polyamines, and betaines. Higher plants and lower plants (as algae) were reported to produce different growth regulators (Mikami et al. 2016; Mori et al. 2017).

3.1 Brassinosteroids (BRs)

They are group of polyhydroxylated steroid growth regulators which have a remarkable role in various biochemical and development processes in different organisms such as plants and algae, including elongation, reproduction, and cell division, in stems and roots, stress responses, leaf senescence, and photomorphogenesis. The most active components of brassinosteroids are termed brassinolide and castasterone which are widely reported in various tissues of seeds, flowers, leaves, pollens, stems, and roots (Bajguz and Hayat 2009). The precursor of brassinolide is the campesterol (C28-sterol) by oxidation at C6 and addition of OH groups into the β-ring. Brassinazole is an inhibitor of brassinosteroid biosynthesis.

Brassinosteroids have been recorded in *Hydrodictyon reticulatum* by Bajguz and Hayat (2009) as well as in angiosperms, gymnosperms, the pteridophyte *Equisetum arvense*, and the bryophyte *Marchantia polymorpha* (Bajguz and Tretyn 2003). Table 14.6 and Fig. 14.13 recorded some seaweed species producing growth substances.

Secondary internode bioassay of beans is used, where brassins cause both cell elongation and cell division as well as bending, swelling, and splitting of the second internode. Brassinosteroids act locally near their site of synthesis and transported in the xylem.

Brassinosteroids have usually effect on the transport of auxin by indirect way (Symons et al. 2008), increase the percentage of ethylene accumulation, and have an additive effect with GA_3, in addition to its synergistic effect with auxins (IAA). Moreover, Brassinosteroids have effects on increase cytokinin and jasmonic acid production and decrease ABA responses. In the seaweed extract of *Ecklonia maxima* (Phaeophyta) called Kelpak (Stirk et al. 2013a, b), auxins, cytokinin, GAs, ABA, and brassinosteroids were detected.

Table 14.6 Seaweed species producing growth substances (growth regulators)

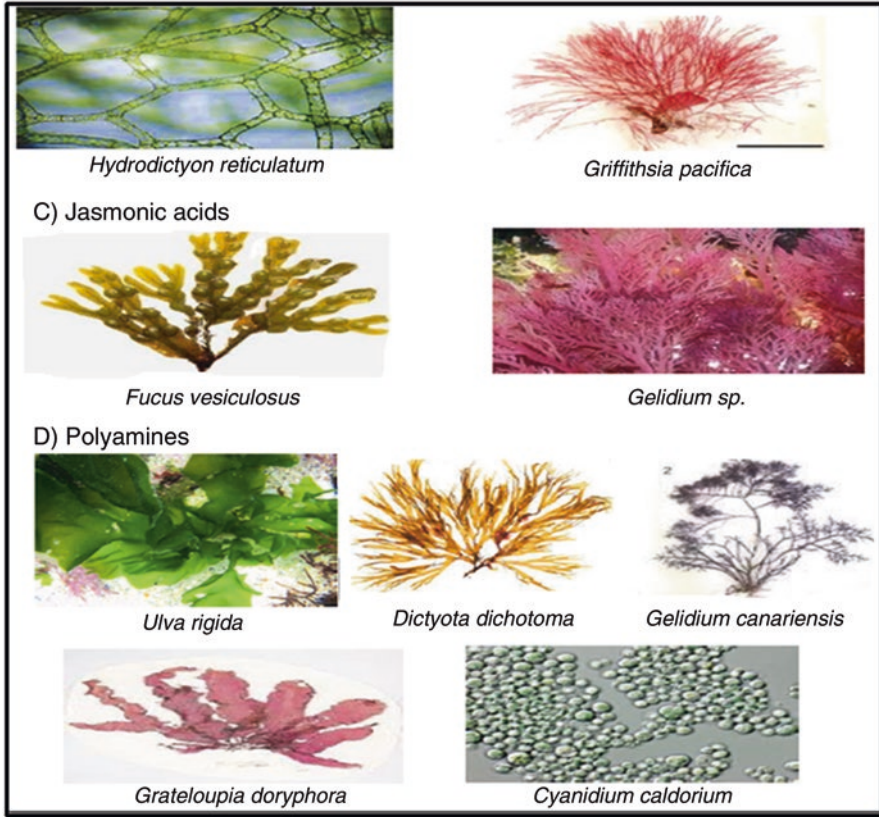| Algal species	Algal group
Brassinosteroids	
Hydrodictyon reticulatum	Chlorophyta
Jasmonic acid	
Fucus vesiculosus	Phaeophyta
Gelidium sp.	Rhodophyta
Polyamines	
Dictyota dichotoma	Phaeophyta
Ulva rigida	Chlorophyta
Gelidium canariensis	Rhodophyta
Grateloupia doryphora	
Cyanidium caldarium	
Rhodomorphin	
Griffithsia pacifica	Rhodophyta

Fig. 14.13 Seaweed species producing growth substances (growth regulators). (**a**) Jasmonic acids. (**b**) Polyamines

3.2 Jasmonic Acid (JA)

Jasmonic acid (is a fatty acid) and its volatile methyl ester (jasmonate) were detected in some seaweed's species. Also, a hydroxylated compound called tuberonic acid, its ME, and its glucosides control potato tuberization development.

Jasmonic acid is produced from the fatty acid linolenic acid (18:3) and plays an important role in plant defense by inducing the synthesis of proteinase inhibitors.

Jasmonates inhibit seed and growth germination and promote abscission, fruit pigmentation, and ripening formation. Jasmonic acid and methyl jasmonate were detected in many species of microalgae and cyanobacteria. Moreover, it was observed in the red seaweed *Gelidium latifolium* and in brown seaweeds (oxilipins and lipoxygenases) as reported by Arnold et al. (2001) shown in Table 14.6 and Fig. 14.13.

3.3 Polyamines (Aliphatic Amines)

Different seaweed species produced polyamines in the red macroalgae *Gelidium canariensis*, *Grateloupia doryphora*, and *Cyanidium caldarium*, in the brown *Dictyota dichotoma*, as well as in the green *Ulva rigida* (Table 14.6 and Fig. 14.13). The content as well as the biosynthesis of polyamines in algae doesn't differ from that in higher plants (50–150 µg/g F.wt).

Polyamine content in macroalgae changes with seasons and developmental stage (Marián et al. 2000; Sacramenta et al. 2004; Alcazar et al. 2010). Polyamines belong to the putrescine group (putrescine, spermine, and spermidine). It derived from the carboxylation of the amino acids, arginine and ornithine, putrescine (diamine) → spermidine (triamine) → spermine (quadramine).

It exerts regulatory control on the development and growth at very low concentration (especially cell division and morphology). In carrot tissue culture, when polyamine content is low, callus growth only occurs, but at higher concentration, the embryo is formed (polyamines are released to the outer growth media). They aren't recorded in the commercial seaweed products till now.

3.4 Salicylic Acid (SA)

It is recognized recently as potential regulatory compound. It is produced from phenylalanine (AA).

Salicylic acid plays a role in the pathogen's resistance. It was reported that SA enhance flower longevity, inhibit biosynthesis of ethylene and seed germination, and reverse the effect of ABA.

Salicylic acid was detected in the red seaweeds *Pyropia yezoensis* and *Bangia fuscopurpurea* (Mori et al. 2017). The precursors of SA are cinnamic acid and benzoic acid. To quantify the amount of SA in an extract, methyl salicylate (*ortho*-anisic acid) can be used as an internal standard (HPLC analysis) (Forcat et al. 2008).

3.5 Signal Peptides

Small molecular weight peptides (as systemin) were found to have regulatory properties in plants which travel in phloem from attacked leaves by herbivore insects to the distant leaves to protect them from insect attack. The traveled signal peptides induce an increase in the production of jasmonic acid and proteinase in the distant leaves for protection.

Signal peptides produced by plants many have a role in:

1. Activation of defensive responses.
2. Cell proliferation promotion.
3. Nodule formation (in legumes).

No known recorded studies that extracted, separated, and identified such signal peptides in seaweeds till now.

3.6 Small RNA Molecules

Recently, many small RNA molecules of single-stranded RNA that consist of 21–22 nucleotides have been identified in plant phloem which may act as transportable signals that regulate gene expression involved in plant defense against viruses. Many of these micro-RNAs (miRNAs) have been identified which means that they may represent a more general means of regulating gene expression.

No such RNA molecules have been identified in seaweeds.

3.7 Rhodomorphin

This regulator was detected in *Griffithsia pacifica* (red alga) following morphogenetic effects in this alga (Table 14.6 and Fig. 14.13). When an intercalary cell in the filament is removed, the basal cell of the filament starts to secrete the regulator rhodomorphin which increases the formation of reparatory cell. Further studies showed that rhodomorphin is a glycoprotein with molecular weight of 14 kDa. Similar glycoproteins were recorded in the green alga *Volvox* sp. where it acts as a pheromone facilitating the gamete adhesion and fusion. So, the function of these glycoproteins in algae was to provide adhesion and fusion of gametes during sexual reproduction.

3.8 Commercial Seaweed Concentrates (or Extracts)

Many reports were published in literature concerning the presence of plant growth hormones in brown, green, and red seaweeds (Crouch and Van Staden 1993; Stirk et al. 2013a, b; Tuhy et al. 2013). Their presence was determined and confirmed by plant bioassays and chromatographic analysis (TLC/LC/MS, HPLC/MS, and GC/MS).

A commercial seaweed concentrate was firstly prepared from the water zone occupied by *Fucus* and *Ascophyllum* sp. which was tested to have cytokinin-like activity due to the presence of isopentenyladenine (ipA).

During the past two decades, utilization of commercial seaweed products increased as natural sources of fertilizers, biostimulants, and soil ameliorants. It improves plant growth is relatively cheap and easy to apply either as soil manure or as foliar spray. It was suggested that bioactive organic compounds in the seaweed concentrate are responsible for the increase in crop yield (Crouch et al. 1992).

Recent researches showed that macroalgae have recorded the ability as a source for products that contain growth regulators and plant hormones and many of the observed effects to treated crops are now attributed to these constituents. The chemical composition of seaweeds revealed that all the major plant nutrients and trace elements are present in marine seaweeds.

Many investigators recently reviewed the presence of antibiotic, antiviral, antibacterial, and antioxidant activities due to the active substances obtained from marine algae. These substances may be responsible for the reduced harmful effects of some plant pathogens (Hamed et al. 2018).

Some of commercial seaweed products (have commercial names):

1. Maxi-crop/Seasol.
2. Algifert/Algimex/Algistim.
3. SM3/Seamac/Marinure.
4. SWC (Kelpak66).

They contain gibberellin-like activity, betaines, ABA, ethylene, and cytokinins. The commercial seaweed extracts use the seaweeds *Ascophyllum nodosum*, *Ecklonia maxima*, *Enteromorpha compressa*, *Durvillaea potatorum*, *Fucus serratus*, *Porphyra perforata*, *Sargassum muticum*, *Laminaria japonica*, *Macrocystis pyrifera*, *Ectocarpus confervoides*, and *Pylaiella littoralis*.

4   Conclusion

From the obtained data, we can conclude that algae (micro and macro) extracts are rich with plant hormones and other growth regulator substances. So we can use these species commercially as growth stimulants in different agricultural sectors. Some of these algal species can be used as organic fertilizer and biofertilizers due to its ability for nitrogen fixation (in case of species with heterocysts) and its content from inorganic chemical substances (such as phosphorus and potassium), in addition to organic substances and hormones. The wider distribution, high adaptability to different cultural factor conditions (biotic and abiotic stresses), and high growth rates led algae to be considered as an attractive feedstock for developing fertilizer and biorefinery products, in addition to the ability of these algal species to increase the accumulation of phytohormones when exposed to different abiotic stress conditions such as salinity, drought, light intensity, chemical substances, etc.

References

Ahmed M, Stal LJ, Hasnain S (2010) Production of indole-3-acetic acid by the cyanobacterium Arthrospira platensis strain MMG-9. J Microbiol Biotechnol 20(9):1259–1265

Arnold TM, Targett NM, Tanner CE, Halch WI, Ferrari KE (2001) Evidence for methyl Jasmonate induced phlorotannin production in Fucus vesiculosus (Phaeophyceae). J Phycol 37:1026–1029

Alcazar R, Altabbella T, Marco F, Bortolotti C, Reymond M, Koncz C, Carrasco P, Tiburcio AF (2010) Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. Planta 231:1237–1249

Bajguz A, Hayat S (2009) The effect of brassinosteroids on the plant responses to environmental stresses. Plant Physiol Biochem 47:1–8

Bajguz A, Tretyn A (2003) The chemical characteristic and distribution of brassinosteroids in plants. Phytochemistry 62:1027–1046

Bently JA (1960) Role of plant hormones in algal metabolism and ecology. Nature 181:1499–1502

Bleecker AB, Kenode H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18

Crouch IJ, Van Staden J (1993) Evidence for the presence of plant growth regulators in commercial seaweed products. Plant Growth Regul 13:21–29

Crouch IJ, Smith MT, Van Staden J, Lewis MJ, Hoad GV (1992) Identification of auxins in a commercial seaweed concentrate. J Plant Physiol 138:590–594

Du Buy HG, Olson RA (1937) The presence of growth regulators during the early development of *Fucus*. Am J Bot 24:609–611

El Shoubaky GA, Salem EA (2009) Biodiversity in Timsah Lake as a biofertilizer source to the economic plants. Egypt J Bot 49:53–69

El Shoubaky GA, Salem EA (2016) Effect of abiotic stress on endogenous phytohormones profile in some seaweeds. Int J Pharm Phytochem Res 8(1):124–134

Forcat S, Bennett MH, Mansfield JW, Grant MR (2008) A rapid and robust method for simultaneously measuring changes in the phytohormones ABA, JA and SA in plants following biotic and abiotic stress. Plant Methods 4:16–23

Garcia-Jimenez P, Robaina RR (2012) Effects of ethylene on tetrasporogenesis in *Pterocladiella capillacea* (Rhodophyta). J Phycol 48:710–715

Garcia-Jimenez P, Brito-Romano O, Robaina RR (2013) Production of volatiles by the red seaweed *Gelidium arbuscula* (Rhodophyta): emission of ethylene and dimethyl sulfide. J Phycol 49:661–669

Gebser B, Pohnert G (2013) Synchronized regulation of different zwitterionic metabolites in the osmoadaption of phytoplankton. Mar Drugs 11:2168–2182

Grennan AK (2008) Ethylene response factors in Jasmonate signaling and defense response. Plant Physiol 146:1457–1458

Hamed SM, Abd EL-Rhman AA, Abdel-Raouf N, Ibraheem IBM (2018) Role of marine macro algae in plant protection and improvement for sustainable agriculture technology. Beni-Suef Univ J Basic Appl Sci 7:104–110

Holopainen IK, Gershenzon J (2010) Multiple stress factors and emission of plant VOCs. Trends Plant Sci 15:176–184

Klee H (2004) Ethylene signal transduction. Moving beyond *Arabidopsis*. Plant Physiol 135:660–667

Kusano T, Berberich T, Tateda C, Takahashi Y (2008) Polyamines: essential factors for growth and survival. Planta 228:367–381

Li T, Wang CG, Miao J (2007) Identification and quantification of indole-3- acetic acid in the kelp Laminaria japonica Areschoug and its effect on growth of marine micro algae. J Appl Phycol 19:479–484

Li XG, Su YH, Zhao XY, Li W, Gao XQ, Zhang XS (2010) Cytokinin over production caused alteration of flower development is partially mediated by CUC2 and CUC3 in Arabidopsis. Gene 450:109–120

Loreto F, Schnitzler JP (2010) Abiotic stress and induced BVOCs. Trends Plant Sci 15:154–166

Mackerness SAH (2000) Plant responses to ultraviolet-B (UV-B:280-320nm) stress. What are the key regulators? Invited review. Plant Growth Regul 32:27–39

Mackinnon SA, Craft CA, Hilty D, Ugate R (2010) Improved method of analysis for betaines in Ascophyllum nodosum and its commercial seaweed extracts. J Appl Phycol 22:489–494

Marián FD, Garcia-Jiménez P, Robaina RR (2000) Polyamines in marine macro algae: levels of putrescine, spermidine and spermine in the thalli and changes in their concentration during glycerol-induced cell growth *in vitro*. Physiol Plant 110:530–534

Maršálek B, Zahradníčková H, Hronková M (1992) Extracellular abscisic acid produced by cyano-bacteria under salt stress. J Plant Physiol 139(4):506–508

Mikami K, Mori IC, Matsuura T, Ikeda Y, Kojima M, Sakakibara H, Hirayama T (2016) Comprehensive quantification and genome survey reveal the presence of novel phytohormone action modes in red seaweeds. J Appl Phycol 28:2539–2548

Mori IC, Ikeda Y, Matsuura T, Hirayama T, Mikami K (2017) Phytohormones in red seaweeds : a technical review of methods for analysis and a consideration of genomic data. Bot Mar 60(2):153–170

Nimura K, Mizuta H (2002) Inducible effects of abscisic acid on sporophyte discs from *Laminaria japonica* Areschoug (Laminariales, Phaeophyceae). J Appl Phycol 14:159–163

Romanenko EA, Kosakovskaya IV, Romanenko PA (2015) Phytohormones of microalgae: biological role and involvement in the regulation of physiological processes. Pt I. auxins, abscisic acid, ethylene. Int J Algae 17(3):275–289

Sacramenta AT, Garcia-Jimenez P, Alcazar R, Tiburcio AF, Robaina RR (2004) Influence of poly-amines on the sporulation of *Grateloupia* (Halymeniaceae, Rhodophyta). J Phycol 40:887–894

Sitnik KM, Musatenko LI, Vosyuk VA, Vedenicheva NP, Generalova VM, Martin GG, Nesterova AN (2003) Gormonal'nii Kompleks roslin i gribiv (Hormonal complex in plants and fungi). Akademperiodika, Kiev

Skibola CF (2004) The effect of *Fucus vesiculosus*, an edible brown seaweed, upon menstrual cycle length and hormonal status in three pre-menopausal women: a case report. BMC Complement Altern Med 4:10–18

Stirk WA, Van Staden J (1997) Comparison of cytokinin and auxin-like activity in some commer-cially used seaweed extracts. J Appl Phycol 8:503–508

Stirk WA, Van Staden J (2006) Seaweed products as biostimulants in agriculture. In: Critchley AT, Ohno M, Largo DB (eds) World seaweed resources. ETI Information Services Ltd., Wokingham, pp 1–32, (DVD ROM)

Stirk WA, Novák O, Strnad M, Van Staden J (2003) Cytokinins in macroalgae. Plant Growth Regul 41:13–24

Stirk WA, Novák O, Hradecká V, Pěnčik A, Rolčik J, Strnad M, Van Standen J (2009) Endogenous cytokinins, auxins and abscisic acid in *Ulva fasciata* (Chlorophyta) and *Dictyota humifusa* (Phaeophyta): towards understanding their biosynthesis and homoeostasis. Eur J Phycol 44(2):231–240

Stirk WA, Balint P, Tarkowská D, Novák O, Strnad M, Ordog V, Van Staden J (2013a) Hormone profiles in microalgae: gibberellins and brassinosteroids. Plant Physiol Biochem 70:348–353

Stirk WA, Tarkowská D, Turecová V, Strnad M, Van Staden J (2013b) Abscisic acid, gibberellins and brassinosteroids in Kelpak R, a commercial seaweed extract made from Ecklonia maxima. J Appl Phycol 26(1):561–567

Stirk WA, Tarkowska D, Turecová V, Strnad M, Van Staden I (2014) Abscisic acid, gibberellins and brassinosteroids in Kelpak®, a commercial seaweed extract made from *Ecklonia maxima*. J Appl Phycol 26:561–567

Sun TP (2010) Gibberellin-GIDI-DELLA: a pivotal regulatory module for plant growth and devel-opment. Plant Physiol 154:567–570

Symons GM, Ross JJ, Jager CE, Reid JB (2008) Brassinosteroid transport. J Exp Bot 59:17–24

Tarakhovskaya ER, Maslov YI, Shishova MF (2007) Phytohormones in algae. J Plant Physiol 54:163–170

Tuhy L, Chowanska J, Chojnacka K (2013) Seaweed extracts as bio-stimulants of plant growth: review. Chemik 67(7):636–641

Weiss D, Ori N (2007) Mechanisms of cross talk between gibberellins and other hormones. Plant Physiol 144:1240–1246

Yamaguchi S (2008) Gibberellin metabolism and its regulation. Annu Rev Plant Biol 59:225–251

Yokoya NS, Stirk WA, Van staden J, Novák O, Tureckova V, Pencik A, Strnad M (2010) Endogenous cytokinins, auxins and abscisic acid in red algae from Brazil. J Phycol 46:1198–1205

Yoshida K, Igarashi E, Mukai M, Hirata K, Miyamoto K (2003) Induction of tolerance to oxida-tive stress in the green alga Chlamydomonas reinhardtii by abscisic acid. Plant Cell Environ 26:451–457