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Review

The Influence of Abiotic Factors on the Induction of Seaweed Callus

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Abstract: Seaweeds are a major source of functional foods, nutraceuticals, and pharmaceuticals. Seaweed can be sustainably harvested through callus culture, which yields homogenous cells and bioproducts under controlled conditions. Callus induction is a crucial early step in callus culture and is influenced by several abiotic factors. This review aims to discuss the influence of abiotic factors on callus induction in seaweeds, a prerequisite for the application and development of seaweed callus culture. We used three online databases (Springer, Science Direct, and Wiley) to search for the literature on seaweed callus induction published between 1987 and 2020. Thirty-three articles for review were identified and analyzed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The analysis covers 56 seaweed species (3% Chlorophyta, 44% Phaeophyta, and 53% Rhodophyta) under various abiotic treatments, including light irradiance (23%), temperature (15%), media type (21%), plant growth regulators (26%), gelling conditions (9%), and other factors (6%). The information on these abiotic factors is intended to be a practical reference and to foster the further study of the callus culture of seaweed. More studies are needed to determine how to maintain and increase callus mass in suspension culture for the industrial production of seaweed and its metabolites.

Keywords: seaweed; callus induction; abiotic factors; micropropagation; tissue culture

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1. Introduction

Seaweeds are a group of marine plant-like organisms that are divided into three groups based on their pigment content: green (Chlorophyta), brown (Phaeophyta), and red (Rhodophyta). They are widely distributed geographically, from tropical to polar regions, with ecoregions ranging from intertidal to submerged zones that are still exposed to sunlight [1,2]. The diversity of environments results in the diversity of seaweeds and their chemical content [3]. In response to harsh environmental stresses, seaweeds synthesize allelochemicals to compete for space, defend against predators and pathogenic microorganisms, and prevent the establishment of epiphytes [4–6]. Compounds in seaweeds possess

biological activities with health-promoting properties related to immunity, skin health, and growth regulation [7]. Macro- and micro-nutrients contained in seaweed, such as polysaccharides, proteins, lipids, vitamins, minerals, pigments, and phenolic compounds, present antioxidant, anti-inflammatory, antimicrobial, antifungal, antitumor, antiviral, neurotrophic factors, and antihypertensive activities [7–13]. Meinita et al. [14] reported that seaweeds contain bioactive compounds against chronic diseases, such as arthritis, cancer, cardiovascular disease, diabetes, and neurodegenerative disease. These properties make seaweed a valuable source of nutraceuticals, pharmaceuticals, and cosmeceuticals [15–17]. The seaweed market is projected to grow 39.8% or USD 24.98 billion from 2021 to 2028. The utilization of seaweed is mainly for food products, and is expected to grow in cosmetics and personal care products due to its antioxidant activity. In addition, seaweed on the market is also used for pharmaceutical, agricultural, and husbandry purposes [18]. Considering the potential of seaweed as a source of biomass in the food, chemical, and pharmaceutical industries, sustainable seaweed cultivation management is essential, a form of which is tissue culture.

Seaweed biotechnology, particularly tissue culture, is becoming a growing research area because of its potential to produce a variety of compounds with diverse biological activities. Tissue culture was initiated on terrestrial plants in 1902, and has been intensively developed with the advent of methods for genetic manipulation, including gene transfer and genome editing [19,20]. Tissue culture provides sustainable production for tissue development and quality improvement. Culturing seaweed tissues in a controlled environment can boost maximum biomass production and stimulate the production of the desired compounds [21]. Tissue culture is especially important for species with insufficient wild stocks and that offer low yields of bioproducts. The challenges imposed by climate change, pollution, and growing demand can potentially be overcome by the use of tissue culture to ensure stock availability. In addition to providing seed stock, it can also increase bioproduct yield through an understanding of the biosynthetic pathways and regulatory mechanisms of bioactive compounds [22].

Tissue culture can be accomplished either by direct regeneration from explant tissues, or indirectly by callus induction. Callus is a mass of unorganized tissue composed of undifferentiated cell lumps. It develops as part of the wound response in plants [23]. Callus culture is commonly used in tissue culture engineering of terrestrial plants for producing homogenous cells and useful products under controlled conditions. Although seaweeds are known as a source of functional foods, nutraceuticals, and pharmaceuticals, the application of seaweed callus culture is not well developed compared to the culture of terrestrial plants. The induction of calli from explants plays an important role in tissue culture as an initial stage of callus proliferation and growth. Callus cultures are generally used to produce cell suspensions [24]. This cell suspension culture can be used to sustainably maintain and increase the production of bioactive compounds [25]. Callus induction is influenced by abiotic factors. Tissue wounding and changes in the physical environment collectively induce calluses in seaweed [26]. Different groups of seaweeds show different callus induction responses to various abiotic conditions. Abiotic factors, such as light irradiance, temperature, media, growth regulators, gelling conditions, osmolality, salinity, and water turbulence, influence callus induction. This brief review summarizes various abiotic factors that affect callus induction in seaweeds. The development of seaweed callus cultures offers the potential for research purposes, as well as for addressing the supply of seed stocks for mariculture and the production of bioactive compounds.

To date, a few articles reviewing tissue culture or the micropropagation of seaweed [27–29] and its bioprocesses [24] have been published. These articles reviewed seaweed tissue culture and its potential in general. The abiotic factors related to callus induction have not been extensively or systematically investigated, although some relevant studies on the influence of abiotic factors on seaweed callus induction have been performed. Hence, this article reviews the studies conducted to evaluate the role of abiotic factors on callus induction in seaweed.

2. Materials and Methods

The existing literature was searched following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines in the Springer, Science Direct, and Wiley online databases [30]. The words "algae" OR "seaweed" and "micropropagation" OR "callus" OR "callus induction OR "callus formation" were used as keywords. The total number of articles found was 2006, which, upon further screening by checking the title and keywords and removing duplicates, decreased to 259.

A total of 90 articles from the set of 259 articles were chosen in the second screening stage based on their abstract content, including 3 additional articles obtained through manual reference tracing. The criterion used in the screening process was the influence of abiotic factors on callus induction. The words "callus," "induction," "light," "temperature," "media," "algae," and "seaweed" were applied as filters. Of the 57 articles excluded after an in-depth observation of the full text, 33 were included in the third screening. Data from 33 articles were analyzed, extracted, and presented in tables and graphs. The systematic search and screening stages for the articles are summarized in Figure 1.

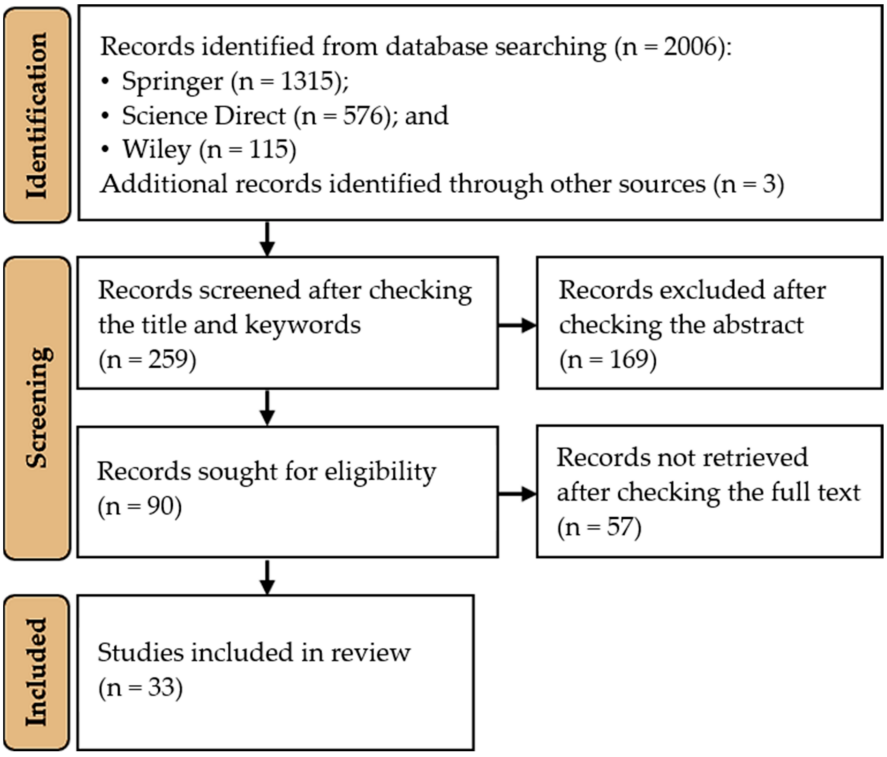

Figure 1. Summary of the search strategy for seaweed callus induction based on PRISMA.

3. Results and Discussion

In total, 2006 articles were screened for eligibility. A set of 33 articles published between 1987 and 2020 were ultimately selected, based on their inclusion of relevant information. Together, in the 33 articles included in this study, 56 species of seaweed were studied, consisting of Chlorophyta 3%, Phaeophyta 44%, and Rhodophyta 53% (Figure 2). *Kappaphycus alvarezii* (11%) and *Saccharina japonica* (9%) were the most studied species with respect to callus induction. *Kappaphycus alvarezi* is a known source of carrageenan and contains bioactive compounds that provide health benefits [31]. Similarly, *Saccharina japonica* is widely utilized as a source of functional food and fucoidan polysaccharides, particularly in East Asia [8,32]. Both seaweeds are economically important; therefore, tissue culture is sought to maintain their productivity and quality [33].

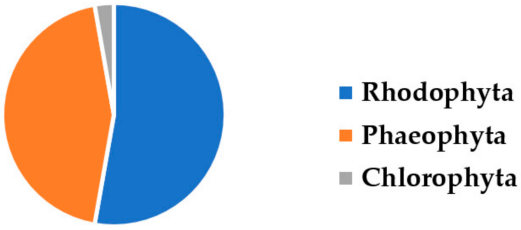

Figure 2. Number of publications on the influence of abiotic factors on callus induction according to taxonomic group.

Studies on seaweed callus induction in this review focused on light (23%), temperature (15%), media (21%), plant growth regulators (PGRs; 26%), gelling conditions of the media (9%), and other factors (6%), all of which can have an effect as abiotic factors on callus induction (Figure 3). Other factors included salinity, osmolality, and the turbulent conditions of the culture media. The treatments related to abiotic factors that were investigated to induce callus are shown in Figure 4.

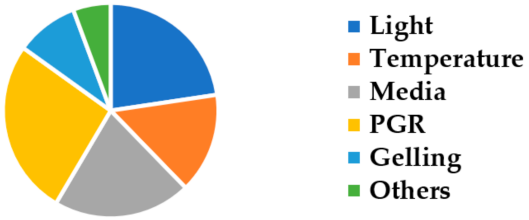

Figure 3. Number of publications on the influence of abiotic factors on callus induction.

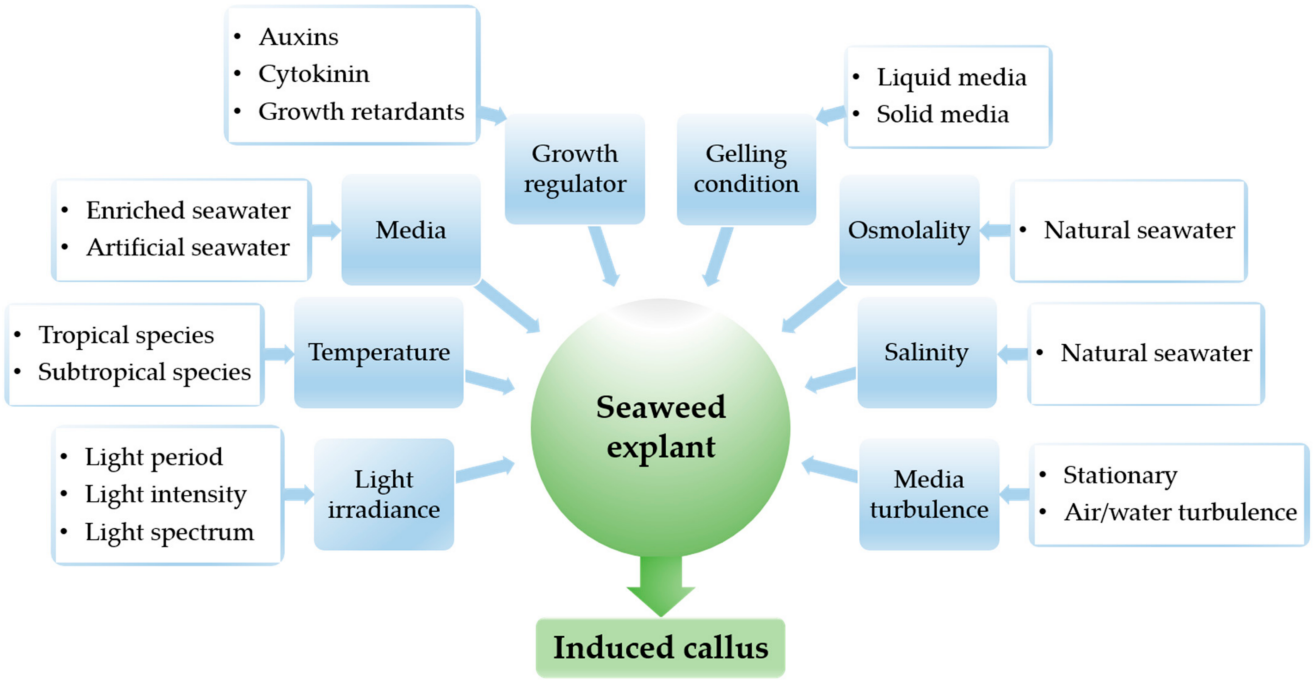

Figure 4. Abiotic factors of seaweed callus induction.

3.1. The Influence of Light

Several studies demonstrated callus induction in seaweed under various light conditions [26,34–45]. Explants from different seaweeds exhibited different callus induction responses to light treatment (Table 1).

Table 1. Studies on the influence of light conditions on the callus induction of seaweeds.

Species	Light Condition	Result	Reference
Costaria costata	13.5, 27, 54, and 108 μmol m^{-2} s^{-1} for 14 h.	Callus was induced optimally at 13.5–27 μmol m^{-2} s^{-1}, and decreased at 108 μmol m^{-2} s^{-1}.	[34]
Ecklonia cava	160 μmol m^{-2} s^{-1} for 0 and 24 h.	Stipe explants induced higher callus in continuous dark (0 h) than continuous light (24 h).	[35]
Ecklonia radiata	0, 3, 10, 20, 30, and 50 μmol m^{-2} s^{-1} for 24 h.	Unpigmented explants induced callus best at 0 μmol m^{-2} s^{-1}; pigmented explants induced callus best at 30 μmol m^{-2} s^{-1}.	[36]
Ecklonia radiata	Dark, red, blue, green, and white light.	Unpigmented explants induced callus best in the dark; pigmented explants induced best in red and white light.	[36]
Eisenia bicyclis	13.5, 27, 54, and 108 μmol m^{-2} s^{-1} for 14 h.	Callus was induced optimally at 13.5 μmol m^{-2} s^{-1}; thereafter, the induction decreased.	[36]
Fucus vesiculosus	35, 45, and 60 μmol m^{-2} s^{-1} for 12, 14, and 16 h.	Only explants treated with 35 μmol m^{-2} s^{-1} for 14 h induced callus.	[37]
Gracilaria corticata	5, 30, and 70 μmol m^{-2} s^{-1} for 12 h.	Explant induced callus (40%) only at 30 μmol m^{-2} s^{-1}.	[26]
Gracilaria domingensis	50 and 150 μmol m^{-2} s^{-1} for 14 h.	Apical segment induced callus (63%) optimally at 50 μmol m^{-2} s^{-1}, but intercalary segment induced callus (81%) optimally at 150 μmol m^{-2} s^{-1}.	[38]
Hizikia fusiformis	7 and 27 μmol m^{-2} s^{-1} for 24 h.	Explant at 27 μmol m^{-2} s^{-1} induced callus better than at 6.75 μmol m^{-2} s^{-1}.	[39]
Hypnea musciformis	5, 30, and 70 μmol m^{-2} s^{-1} for 12 h.	Explant induced callus at 5 and 30 μmol m^{-2} s^{-1} by 10%.	[26]
Kappaphycus alvarezii	5, 30, and 70 μmol m^{-2} s^{-1} for 12 h.	Both 5 and 30 μmol m^{-2} s^{-1} were suitable, but 70 μmol m^{-2} s^{-1} caused bleaching.	[40]
Kappaphycus striatus	5 μmol m^{-2} s^{-1} for 12 h.	Explants induced callus 54–61%.	[41]
Kjellmaniella crassifolia	0, 10, 20, 40, and 80 μmol m^{-2} s^{-1} for 14 h.	Highest growth rate of callus at 10 μmol m^{-2} s^{-1}.	[42]
Saccharina japonica	White, red, and blue light.	Explants induced callus higher in red light (85.9%) than in white (48.0%) and blue (43.0%) lights.	[43]
Saccharina japonica	13.5, 27, 54, and 108 μmol m^{-2} s^{-1} for 14 h.	Callus was induced optimally at 13.5 μmol m^{-2} s^{-1}; an amount exceeding this caused the induction to decrease, and at 108 μmol m^{-2} s^{-1}, the callus was not induced.	[34]
Saccharina japonica	0, 15, 30, 60, and 120 μmol m^{-2} s^{-1} for 12 h.	0–30 μmol m^{-2} s^{-1} induced callus (50–76%); optimal at 30 μmol m^{-2} s^{-1}. Induction decreased at ≥60 μmol m^{-2} s^{-1}.	[44]
Sargassum horneri	White, red, and blue light.	Explants induced callus better in blue light (45%) than in white (42.5%) and red (27.5%) lights.	[45]
Sargassum horneri	20, 80, and 200 μmol m^{-2} s^{-1} for 14 h.	Highest callus induction rate of 50% at 20 μmol m^{-2} s^{-1}.	[45]
Sargassum tenerrimum	5, 30, and 70 μmol m^{-2} s^{-1} for 12 h.	Explant induced callus (10%) only at 30 μmol m^{-2} s^{-1}.	[26]
Turbinaria conoides	5, 30, and 70 μmol m^{-2} s^{-1} for 12 h.	Explant induced callus (40%) only at 30 μmol m^{-2} s^{-1}.	[26]
Undaria pinnatifida	13.5, 27, 54, and 108 μmol m^{-2} s^{-1} for 14 h.	Callus was induced optimally at 13.5–27 μmol m^{-2} s^{-1}, after which the induction decreased, and at 108 μmol m^{-2} s^{-1}, the callus was not induced.	[34]

Three different types of light treatment were applied for callus induction in seaweed: (1) light intensity, (2) light period, and (3) light spectrum. The light intensity ranged from 0 (dark) to 150 μmol m^{-2} s^{-1}, but most of the seaweeds induced calli optimally at low light intensities of 0–35 μmol m^{-2} s^{-1}. The callus induction of seaweed explants decreased at a high light intensity [34,42,44,45], and the explants bleached (turned white) and died [26,40]. Previous studies have shown that the light period for callus induction was typically 12–14 h. Zayed et al. [37] reported that *Fucus vesiculosus* induced calli better in the 14 h lighting period than at 12 and 16 h. In olives, a higher plant, callus induction occurred at a higher rate at a light intensity of 500–1000 lx (≈7–14 μmol m^{-2} s^{-1}) than at 2000–2500 lx (≈28–35 μmol m^{-2} s^{-1}) or in dark conditions (0 μmol m^{-2} s^{-1}) [46]. Light irradiance may cause stress in explants when continuously exposed [47]. Kawashima and Tokuda [35] demonstrated that *Ecklonia cava* exposed to continuous light at 160 μmol m^{-2} s^{-1} showed an induction of fewer calluses than those exposed to dark conditions. Moreover, in *E. radiata* unpigmented explants, callus induction occurred better in the dark than under continuous light at 3–50 μmol m^{-2} s^{-1}, although pigmented explants induced callus optimally at 30 μmol m^{-2} s^{-1} [36]. According to Staehr and Wernberg [48], *E. radiata* can change

the pigment and photoprotective pigment ratio in tissues in response to changes in light intensity. *Kjellmaniella crassifolia* and *Saccharina japonica* also showed optimum callus induction in the presence of light irradiance (10 and 30 μmol m^{-2} s^{-1}) compared to dark conditions [42,44]. This might explain why pigmented explants require light to induce callus formation. Tabuchi et al. [44] suggested that callus cells can develop autotrophically. Hwang et al. [39] reported that continuous irradiation of *Hizikia fusiformis* resulted in a higher callus induction at 27 μmol m^{-2} s^{-1} than at 7 μmol m^{-2} s^{-1}. However, *H. fusiformis* calli maintained their undifferentiated form at a lower light intensity and differentiated into plantlets at 27 μmol m^{-2} s^{-1} after 30 days of culture. This result can be considered for maintaining the callus form in tissue culture or continuing its development into seaweed.

Studies on the effects of the wavelength of light on seaweed callus induction are limited to three species: *E. cava*, *S. japonica*, and *Sargassum horneri* [36,43,45]. Three light spectra were observed to induce callus formation in *S. japonica* and *Sargassum horneri*: white light, blue light (400–530 nm), and red light (600–700 nm). For *E. cava*, green light (530 nm) was used as an additional treatment. Both Laminariales, *E. cava*, and *S. japonica* showed a high callus induction under red light treatment. In contrast, the callus induction in *S. horneri* was low under red-light irradiance and optimal under blue light. The proper light spectrum for the callus induction of *S. horneri* corresponds to that for the culture of its germling and immature stages, for which it has been shown that the growth rate is fastest under blue light irradiance [49]. In *S. japonica*, red light promotes vegetative propagation by inducing callus formation and accelerating holdfast and stipe growth. Red-light treatment might be related to indoleacetic acid (IAA) metabolism in the tissue during vegetative propagation [43].

3.2. The Influence of Temperature

Several studies have been published on the influence of temperature on callus induction in seaweeds. The most frequently reported seaweeds in these studies are *Costaria costata* [34], *Ecklonia cava* [35], *Eisenia bicyclis* [34], *Kjellmaniella crassifolia* [42], *Saccharina japonica* [34,44], and *Undaria pinnatifida* [34,35]. A summary of the influence of temperature on callus induction in seaweeds is presented in Table 2.

Table 2. Studies on the influence of temperature on callus induction in seaweeds.

Species	Temperature Condition	Result	Reference
Ceramium kondoi	12–15, 18–20, and 26 °C	Highest callus induction rate 2% at 12–15 °C.	[50]
Costaria costata	10, 15, 20, and 25 °C	Callus was induced optimally at 15 °C. At 25 °C, callus gradually died.	[34]
Ecklonia cava	8, 13, 18, and 23 °C	Meristem explants induced callus at 13 °C (86%) better than at 18 °C (29%). Stipe explants induced callus better at temperatures ranging from 8–13 °C.	[35]
Eisenia bicyclis	10, 15, 20, and 25 °C	The induced callus increased with increasing temperature, with an optimum at 20 °C. At 25 °C, the induction decreased.	[34]
Furcellaria fastigiata	12–15, 18–20, and 26 °C	Highest callus induction rate 16% at 18–20 °C.	[50]
Gelidium vagum	12–15, 18–20, and 26 °C	Highest callus induction rate 15% at 18–20 °C.	[50]
Gracillaria verrucosa	12–15, 18–20, and 26 °C	Highest callus induction rate 4% at 18–20 °C.	[50]
Kappaphycus striatus	24 ± 2 °C	Explants induced callus 54–61%.	[41]
Kjellmaniella crassifolia	5, 10, 15, 20, and 25 °C	Explant induced callus and grew at 5–15 °C, highest growth at 10 °C. At 20–25 °C, explants died.	[42]
Phyllophora nervosa	12–15, 18–20, and 26 °C	Highest callus induction rate 18% at 18–20 °C.	[50]
Saccharina japonica	10, 15, 20, and 25 °C	Callus was induced optimally at 15 °C. Explants died at 25 °C.	[34]
Saccharina japonica	5, 10, 15, and 20 °C	Callus was induced optimally at 10 °C. Increasing temperature higher than 10 °C decreased callus induction.	[44]
Sargassum horneri	5, 20, and 30 °C	Highest callus induction rate 37.5% at 20 °C.	[45]
Undaria pinnatifida	8, 13, and 18 °C	Stipe induced callus better at 13–18 °C (20%), compared with 8 °C (10%). Meristem induced best at 13 °C (33%), compared with 8 and 18 °C (20 and 22%, respectively).	[51]
Undaria pinnatifida	10, 15, 20, and 25 °C	Callus was induced optimally at 15 °C. Explants died at 25 °C.	[34]

Temperatures ranging from 8 to 26 °C were observed to induce callus formation in seaweeds. According to these studies, the callus is optimally induced at temperatures of 10–20 °C [34,35,42,44,45,50], and temperatures higher than 20 °C cause a decrease in callus

induction and even mortality of the explants [34,44]. There may be a correlation between light radiation and temperature for both callus formation and explant survival. Notoya et al. [34] reported that increasing the intensity of light incident on *S. japonica* explants at a temperature of 15 °C decreased callus development and killed the explants. The explants survived at a lower temperature of 10 °C, although callus growth continued to diminish with increasing light intensity.

Kappaphycus striatus is a seaweed that survives and in which calli formation is induced at temperatures over 20 °C [41]. This is conceivably because *K. striatus* inhabits the tropics, where the optimal temperature for photosynthesis is 31–32 °C [52,53]. Other seaweeds that show reduced callus induction and perish at temperatures of 20–25 °C are known as subtropical species, namely *C. costata*, *E. bicyclis*, *K. crassifolia*, *S. japonica*, and *U. pinnatifida* [54–58]. Temperatures below 20 °C are optimal for the development of these subtropical species. As a result, in tissue culture, suboptimal temperatures might hinder callus induction and even death. Considering that the frequency and rate of callus development are affected by the incubation temperature, it is essential to evaluate the temperature of the native environment as well as the optimal conditions for seaweed growth prior to callus induction.

3.3. The Influence of Media Types

Callus induction in seaweeds has been observed in various media types, including seawater, TC-1; Murashige and Skoog (MS); modified MS, ASP 12, ASP-12-NTA; modified ASP-12-NTA with calcium, ASP-C-1, ASP-6-F2; Conwy (CW); 50% Von Stosch (VS); Guillard and Ryther (f/2); modified f/2; enriched seawater (ESW); modified ESW, SWA, SWII, SWII with cholesterol (SWIICH); Provasoli enriched seawater (PES); PES with iodine (PESI); and some modified PES with carbon and vitamin sources [23,35,36,40,41,51,59–63]. The callus induction in seaweeds in these media is summarized in Table 3.

Table 3. Studies on the influence of media type on callus induction in seaweeds.

Species	Media Type	Result	Reference
Cytoseira osmundacea	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in MS medium, but induced in seawater (10%), PES (9%), TC-1 (11%), ASP-C-1 (4%), ASP-12-NTA (2%), and ASP-6-F2 (4%).	[23]
Ecklonia cava	SWA, SWII, SWIICH, PESI, ASP 12, and ASP-C-1.	Stipe and meristem did not induce callus in ASP-C-1. In other media, the callus was induced without any particular media preference.	[35]
Ecklonia radiata	0.7% agar PES medium supplemented with different carbon sources (1 and 10 mM): arabinose, aspartic acid, glyceric acid, glycerol, mannitol, mannose, and sodium pyruvate.	Growth of callus of unpigmented cells was inhibited in the dark by aspartic acid (1 and 10 mM), mannitol, or glycerol (10 mM); other carbon sources had no effect. In light, all the carbon sources stimulated the callus growth. In pigmented cells, only glycerol showed a small stimulatory effect.	[36]
Ecklonia radiata	0.7% agar PES medium supplemented with different vitamin sources (0, 0.1, 1, 10, 100, and 1000 μg L^{-1}): biotin, thiamine HCl, nicotinic acid, cyanocobalamin, pyridoxine HCl, and tocopherol.	Nicotinic acid or thiamine (1 μg L^{-1}) or biotin (10–100 μg L^{-1}) supported callus growth of unpigmented cells. Other vitamins had no effect.	[36]
Egregia menziesii	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in TC-1 and MS, but was induced in seawater (5%), PES (9%), ASP-C-1 (1%), ASP-12-NTA (7%), and ASP-6-F2 (1%).	[23]
Enteromorpha intestinalis	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus induced in all media 83–91%. Highest rate in seawater.	[23]
Eucheuma uncinatum	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in seawater and ASP-C-1, but was induced in PES (3%), TC-1 (1%), MS (1%), ASP-12-NTA (3%), and ASP-6-F2 (2%).	[23]
Gelidium robustum	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in seawater and TC-1, and ASP-12-NTA, but was induced in PES (2%), MS (1%), ASP-C-1 (0.3%), and ASP-6-F2 (0.5%).	[23]

Table 3. *Cont.*

Species	Media Type	Result	Reference
Gigartina exasperata	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in MS, ASP-C-1 and ASP-6-F2, but was induced in seawater (1%), PES (3%), TC-1 (1%), and ASP-12-NTA (1%).	[23]
Gracilaria papenfussii	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in seawater and TC-1, and ASP-C-1 medium, but was induced in PES (0.5%), MS (0.6%), ASP-12-NTA (2%), and ASP-6-F2 (0.3%).	[23]
Grateloupia filicina	f/2, f/2 + glycerol (0.5, 1.0, and 1.5%), and f/2 + NAA or BAP or kinetin (10^{-5}, 10^{-6}, and 10^{-7} M) in 1.5% agar.	Explants only induced callus in f/2 (30%), f/2 + glycerol 0.5% (70%), f/2 + glycerol 1.0% (10%), f/2 + NAA 10^{-5} M (40%), f/2 + NAA 10^{-6} M (25%), and f/2 + BAP 10^{-6} M (30%).	[59]
Grateloupia filicina	PES, PES + glycerol (0.5, 1.0, and 1.5%), and PES + NAA or BAP or kinetin (10^{-5}, 10^{-6} and 10^{-7} M) in 1.5% agar.	Explants only induced callus in PES (20%), PES + glycerol 0.5% (25%), PES + glycerol 1.0% (10%), PES + glycerol 1.5% (10%), PES + NAA 10^{-5} M (25%), PES + NAA 10^{-6} M (10%), and PES + kinetin 10^{-5} M (5%).	[59]
Grateloupia filicina	ESW, ESW + glycerol (0.5, 1.0, and 1.5%), and ESW + NAA or BAP or kinetin (10^{-5}, 10^{-6} and 10^{-7} M) in 1.5% agar.	Explants only induced callus in ESW (10%)	[59]
Kappaphycus alvarezii	CW medium in 0.8% agar.	Explants induced callus 40%.	[60]
Kappaphycus alvarezii	PES medium in 1.5% agar.	Explants induced callus 82%.	[40]
Kappaphycus alvarezii	50% VS, f/2, and ASP-12-NTA in 0.5% agar.	Explants induced callus highest in 50% VS (>95%) and lowest in ASP-12-NTA (~35%). In f/2 medium, induced callus was up to 82%.	[61]
Kappaphycus alvarezii	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus induced in all media 0.8–10%. Highest and lowest rates in PES and ASP-6-F2, respectively.	[23]
Kappaphycus striatus	PES medium in 1.5% agar.	Explants induced callus 54–61%.	[41]
Laminaria sinclairii	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in ASP-C-1 medium, but was induced in seawater (28%), PES (23%), TC-1 (9%), MS (4%), ASP-12-NTA (3%), and ASP-6-F2 (25%).	[23]
Macrocystis pyrifera	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in ASP-C-1 medium, but was induced in seawater (30%), PES (35%), TC-1 (11%), MS (2%), ASP-12-NTA (14%), and ASP-6-F2 (20%).	[23]
Pelvetia fastigiata	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in MS, ASP-C-1, and ASP-6-F2 medium, but was induced in seawater (20%), PES (21%), TC-1 (18%), and ASP-12-NTA (6%).	[23]
Porphyra lanceolata	MS, ASP-C-1, ASP 12 NTA, or ASP-6-F2 in 1.5% agar medium.	Callus induced in all media 75–84%. Highest rate in ASP-6-F2 medium.	[23]
Porphyra nereocystis	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus induced in all media 70–90%. Highest and lowest rates in ASP-12-NTA and ASP-6-F2, respectively.	[23]
Porphyra perforata	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus induced in all media 76–89%. Highest rate in ASP-12-NTA.	[23]
Prionitis lanceolata	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in seawater, TC-1, MS, and ASP-6-F2 medium, but was induced in PES (1%), ASP-C-1 (0.8%), and ASP-12-NTA (0.3%).	[23]
Saccharina japonica	MS-S-V, PESI, and ASP-C-1 in 1.5% agar medium and MS liquid medium.	Callus induced only in MS-S-V (67.3%) and PESI (75.5%).	[62]
Saccharina japonica	ASP-12-NTA in 0.5% agar with various Ca^{2+} concentrations (2.5–15 mM).	5 mM of Ca^{2+} supplementation was the highest callus induction rate (46.3%); a concentration higher than this decreased its induction rate.	[63]
Sargassum fluitans	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in ASP-6-F2, but was induced in seawater (15%), PES (12%), TC-1 (12%), MS (2%), ASP-C-1 (1%), and ASP-12-NTA (4%).	[23]
Sargassum muticum	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus was not induced in ASP-C-1, but was induced in seawater (25%), PES (27%), TC-1 (23%), MS (0.7%), ASP-12-NTA (5%), and ASP-6-F2 (7%).	[23]
Smithora naiadum	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus induced in all media 5–85%. Highest and lowest rates in seawater and ASP-6-F2, respectively.	[23]
Ulva angusta	Seawater, PES, TC-1, MS, ASP-C-1, ASP-12-NTA, or ASP-6-F2 in 1.5% agar medium.	Callus induced in all media 80–92%. Highest rate was in MS.	[23]
Undaria pinnatifida	SWA, SWII, PESI, and ASP 12.	Stipe and meristem induced callus best in PESI (20 and 30%, respectively).	[51]

The use of appropriate media can support callus induction and the formation of a sufficient mass for further tissue culture. A sufficient callus mass is also required for biochemical, physiological, and genetic research [50]. Polne-Fuller and Gibor [23] revealed that the calluses of some seaweeds, such as *Cytoseira osmundacea*, *Enteromorpha intestinalis*, *Laminaria sinclairii*, *Pelvetia fastigiata*, *Sargassum fluitans*, *S. muticum*, and *Smithora naiadum*, did not require media-rich nutrients and were induced at a higher rate in unsupplemented seawater than in enriched media. In some species of *Egregia menziesii*, *Eucheuma uncinatum*, *Gelidium robustum*, *Gigartina exasperata*, *Gracilaria papenfussii*, *Kappaphycus alvarezii*, *Macrocystis pyrifera*, *Porphyra nereocystis*, *P. perforata*, and *Prionitis lanceolata*, callus formation was induced in enriched media, such as PES and ASP-12-NTA. Several studies used PES as the basal medium, which is enriched with various carbons, vitamins, plant growth hormones, and iodine (PESI). Lawlor et al. [36] reported that PES supplemented with arabinose, aspartic acid, glyceric acid, glycerol, mannitol, mannose, and sodium pyruvate stimulated the callus growth of unpigmented cells of *E. radiata* under light-irradiated conditions, whereas under dark conditions, carbon sources (1–10 mM of aspartic acid, 10 mM of mannitol, or glycerol) had no effect or even inhibited its growth. Glycerol showed the opposite effect on pigmented cells, although stimulation was minimal. The stimulatory effect of glycerol in PES media was also reported by Baweja and Sahoo [59]. *Grateloupia filicina* in PES with 0.5% glycerol exhibited more effective callus induction than in PES, but the rate of induction decreased with increasing the glycerol concentration (1.0–1.5%). The supplementation of f/2 media with glycerol also increased callus induction in *G. filicina*, but the supplementation of ESW media did not [59]. Glycerol in the ASP-6-F2 medium had no effect on *Gracilaria verrucosa* callus induction until plant growth regulators were introduced [64].

The brown seaweed *E. radiata* exhibited the callus induction in PES media supplemented with vitamins. Among biotin, thiamine, nicotinic acid, cyanocobalamin, pyridoxine, and tocopherol (at various concentrations of 0–1000 μg L^{-1}), only nicotinic acid, thiamine (1 μg L^{-1}), and biotin (10–100 μg L^{-1}) stimulated the callus growth of unpigmented cells [36]. PES media contains 0.2 μg L^{-1} cyanocobalamin, 0.1 μg L^{-1} biotin, and 10 μg L^{-1} thiamine [65]; therefore, raising the amounts of thiamine and biotin, as well as nicotinic acid, offers excellent conditions for *E. radiata* callus induction.

Media ASP-12-NTA is also frequently used for callus induction in seaweeds and shows good results. Several studies modified this medium to ASP-6-F2, ASP-C-1, and ASP-12-NTA with calcium [63,66,67]. The nitrilotriacetic acid (NTA) content of these media is distinctive, in comparison to that of the other media mentioned above. NTA can act as a chelator and toxin in seaweeds [35]. Polne-Fuller and Gibor [23] found that the PES medium was more effective than ASP at inducing callus formation in a variety of seaweeds. PES and ASP (ASP-12-NTA and their modification) media have a fundamental difference because PES consists of enriched natural seawater, whereas ASP-based media consist of artificial seawater. The utilization of artificial seawater can be considered when demonstrating tissue cultures that are free of undesired materials and substances from natural waters. Kanamori et al. [63] showed that the addition of 5 mM calcium (Ca^{2+}) to ASP-12-NTA increased callus induction in *Saccharina japonica*. The presence of Ca^{2+} in the media enhances intracellular Ca^{2+}, which results in the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. NADPH oxidase produces radical oxygen species (ROS), induces the elongation of callus-like cells in explants, and promotes filamentous callus-like cells.

3.4. The Influence of Plant Growth Regulators

Plant growth regulators (PGRs) can be applied at specific growth stages to influence plant development. PGRs are widely used in tissue cultures of higher plants [68–70]. Synthetic as well as natural PGRs have been utilized for seaweed callus induction, namely, 2,4-dichlorophenoxyacetic acid (2,4-D); picloram (PIC); phenylacetic acid (PAA); indole-3-acetic acid (IAA); indole-3-butyric acid (IBA); α-naphthaleneacetic acid (NAA); 6-benzylaminopurine (BAP); 2-Isopentenyladenine (2iP); kinetin (KIN); uniconazole (UNI); N-(2-chloro-4-pyridyl)-N′-phenylurea (CPPU); gibberellic acid (GA); 5-aminolevulinic acid (ALA); zeatin (ZEA);

and the polyamine spermine [40,44,45,60,71–81]. The effect of PGRs on callus induction in seaweed is summarized in Table 4.

Table 4. Studies on the activity of plant growth regulators on callus induction in seaweeds.

Species	PGR Treatment	Result	Reference
Chondracanthus chamissoi	0.5–50 µM of IAA, 2,4-D and BAP.	Highest callus induction (6–8%) with BAP 50 µM in the apical segment, but IAA 50 µM showed the highest callus induction rate (18–20%) in the intercalary segment.	[71]
Eucheuma denticulatum	Combination of NAA or PAA (0–10 mg L^{-1}) and 2iP (0–1 mg L^{-1}) or BAP (1 mg L^{-1}).	0.1–1.0 mg L^{-1} of NAA and 1.0 mg L^{-1} of BAP induced callus, but explants were killed by 10 mg L^{-1} of NAA.	[72]
Gracilaria perplexa	0.1–100.0 µM of IAA, 2,4-D and KIN.	1 µM of IAA, 2,4-D or kinetin was optimal in increasing the callus induction of intercalary segments.	[73]
Gracilaria tenuistipitata	0.1–100.0 µM of IAA, 2,4-D and KIN.	100.0 µM of IAA or 2,4-D optimally induced callus on the apical segments (>80%). Kinetin decreased the induction rate at higher concentrations.	[73]
Gracilaria vermiculophylla	0.1 and 1.0 mg L^{-1} of IAA, 0.1, 1.0 and 10.0 mg L^{-1} of 2,4-D, 0.1 and 1.0 mg L^{-1} of BAP, and the combinations 0.1 + 0.1, 0.1 + 1.0, 1.0 + 0.1 and 1.0 + 1.0 mg L^{-1} of IAA + BAP.	0.1 mg L^{-1} of IAA; 0.1 mg L^{-1} of 2,4-D; and 1 mg L^{-1} of BAP were the best for callus growth in apical segments. In the intercalary segments, 0.1 mg L^{-1} of IAA, 10.0 mg L^{-1} of 2,4-D, and the combinations 0.1 + 0.1, 0.1 + 1.0 and 1.0 + 0.1 (IAA + BAP) were optimum for callus growth.	[74]
Gracilariopsis tenuifrons	0.5 and 5.0 mg L^{-1} of IAA, 0.5 and 5.0 mg L^{-1} of BAP, and 1:1, 1:5 and 5:1 mg L^{-1} of IAA:BAP.	0.5 mg L^{-1} of IAA and 5:1 mg L^{-1} of IAA:BAP showed the highest callus formation in apical segments. A total of 5.0 mg L^{-1} of IAA stimulated the highest callus formation in intercalary segments.	[75]
Grateloupia dichotoma	0.5 and 5.0 mg L^{-1} of IAA, 0.5 and 5.0 mg L^{-1} of 2,4-D, 0.5 and 5.0 mg L^{-1} of BAP, 1:5 and 5:1 mg L^{-1} of IAA:BAP.	All PGRs induced callus, but the maximal induction was using 2,4-D or IAA:BAP (1:5 mg L^{-1}).	[76]
Kappaphycus alvarezii	0–25 mg L^{-1} of 2,4-D in solid PES (1%).	5 mg L^{-1} of 2,4-D induced the highest rate of callus formation (50%).	[77]
Kappaphycus alvarezii	Combination of 2,4-D (0.1 and 1.0) and KIN (0.1 and 1.0 mg L^{-1}).	0.1 mg L^{-1} of 2,4-D and 1 mg L^{-1} of kinetin induced the highest rate of callus formation (40.7%).	[77]
Kappaphycus alvarezii	0.5–1 mg L^{-1} of BAP and 0.5–1 mg L^{-1} of NAA in solid CW (0.8%).	Explants induced callus 10–60%.	[60]
Kappaphycus alvarezii	2.5–5 mg L^{-1} of IAA in solid CW (0.8%).	Explants induced callus 0–20%.	[60]
Kappaphycus alvarezii	2.5–5 mg L^{-1} of IAA in solid PES (0.8%).	Explants induced callus 40–50%.	[60]
Kappaphycus alvarezii	0.5–1 mg L^{-1} of BAP in solid PES (0.8%).	Explants induced callus 30–70%.	[60]
Kappaphycus alvarezii	1 mg mL^{-1} of BAP and 2.5 mg L^{-1} of IAA in solid CW and PES (0.8%).	Explants induced callus 30–50%.	[60]
Kappaphycus alvarezii	0.5 mg mL^{-1} of BAP and 0–1 mg L^{-1} of NAA in solid PES (0.8%).	Explants induced callus 40–50%.	[60]
Kappaphycus alvarezii	0.5–1 mg L^{-1} of NAA in solid CW and PES (0.8%).	Explants induced callus 20–50%.	[60]
Kappaphycus alvarezii	0.1–1 mg L^{-1} of NAA or BAP in solid PES media.	Explants induced callus 84%.	[40]
Kappaphycus alvarezii	Combination of NAA or PAA (0–10 mg L^{-1}) and 2iP (0–1 mg L^{-1}) or BAP (1 mg L^{-1}).	The presence of 2iP induced callus. Increasing NAA to 1 mg mL^{-1} favored callus induction. The combination of NAA or PAA (1 mg L^{-1}) and BAP (1 mg L^{-1}) was as effective as NAA and 2iP in inducing callus. NAA and PAA at higher concentration (10 mg L^{-1}) decreased the callus induction rate.	[72]
Kappaphycus alvarezii	Sixteen combinations of NAA (0 or 1 mg L^{-1}), KIN (0 or 1 mg L^{-1}), BAP (0 or 1 mg L^{-1}), and spermine (0 or 0.0018 mg L^{-1}).	NAA or BAP at 1 mg L^{-1} induced calluses at a 129% higher rate than the control. The combination of NAA, kinetin, and spermine (1, 1, and 0.018 mg L^{-1}, respectively) increased callus induction 85% higher than the control.	[78]
Laminaria digitata	2,4-D (0.45–45 µM), NAA (0.53–53 µM), PIC (0.04–4 µM)/BAP (0.44 µM), NAA (0.53 µM)/CPPU (0.04–4 µM), PIC (4 µM)/KIN (0.46–2.3 µM), PIC (4 µM)/CPPU (0.4–2 µM), PIC (4 µM)/ZEA (0.45–0.9 µM), PIC (4 µM)/CPPU (0.45–0.9 µM).	High callus induction (38–50%) in media containing 2,4-D alone (0.45–45 µM). The combination of Pi (4 µM)–CPPU (2 µM) and CPPU (4 µM)–NAA (0.54 µM) also induced callus at a high rate (50 and 29.5%, respectively).	[79]

Table 4. *Cont.*

Species	PGR Treatment	Result	Reference
Pelvetia siliquosa	1, 2, 3, 4, and 5 mg L^{-1} of IAA	Callus induced (90%) optimally at an IAA concentration of 1–3 mg L^{-1}. More than 3 mg L^{-1} of IAA drastically decreased callus induction (~5%).	[80]
Saccharina japonica	0, 1, 5, 10, 50, 50, 100, and 500 mg L^{-1} of ALA.	100 mg L^{-1} of ALA was optimal to induce callus in apical (58.2%), middle (94.1%), and basal (100%) segments.	[44]
Sargassum horneri	IAA, 2,4-D, BAP, GA, and 5–10 µM of UNI.	IAA, 2,4-D, BAP, and GA treatments did not induce calluses, but 5 µM of uniconazole induced the highest number of calluses.	[45]
Sargassum polycystum	4.52–22.62 µM of 2,4-D, 5.71–28.54 µM of IAA, 4.92–24.60 µM of IBA, 5.37–26.85 of NAA, 1.00–10.00 µM of PIC, 4.44–22.20 µM of BAP, 4.65–23.23 µM of KIN, 1.00–10.00 CPPU, 1.00–10.00 µM of UNI.	Calluses were only induced in media containing kinetin at 4.65–13.94 µM (11.11–14.81%) or UNI 3–10 µM (14.81–22.22%). UNI at 7 µM caused the highest induction rate (22.22%) of all treatments.	[81]

Notes: 2,4-dichlorophenoxyacetic acid (2,4-D); picloram (PIC); phenylacetic acid (PAA); indole-3-acetic acid (IAA); indole-3-butyric acid (IBA); α-naphthaleneacetic acid (NAA); 6-benzylaminopurine (BAP); 2-Isopentenyladenine (2iP); kinetin (KIN); uniconazole (UNI); N-(2-chloro-4-pyridyl)-N′-phenylurea (CPPU); gibberellic acid (GA); 5-aminolevulinic acid (ALA); and zeatin (ZEA).

To optimize tissue culture, analyzing the effect of PGRs on seaweed callus induction is important. In comparison to other abiotic variables, the effect of PGRs on callus induction in seaweed has only recently been intensively studied. Of the 14 published articles, >40% discussed the use of IAA, BAP, 2,4-D, and NAA as PGRs. The supplementation of media with IAA, 2,4-D, BAP, and the combination of IAA and BAP has been shown to optimally prime seaweeds of the Gracilariaceae family for callus induction [73–75]. KIN also induced callus formation in *G. perplexa*, but had a negative effect on *G. tenuistipitata* [73]. IAA supplementation of 1–3 mg L^{-1} in PESI solid media has also been reported to induce callus production up to 90% in brown seaweed *Pelvetia siliquosa*, but at higher concentrations of 4 and 5 mg L^{-1}, the induction rate drops to 50 and 5%, respectively [80].

Sulistiani et al. [60] reported that IAA supplementation (2.5–5.0 mg L^{-1}) in PES media (40–50%) induced the callus of *K. alvarezii* at a higher rate than in CW media (0–20%). However, the highest callus induction of this seaweed (84%) was obtained from 0.1–1 mg L^{-1} NAA or BAP in solid PES media. Dawes and Koch [72] also showed that 1 mg L^{-1} NAA or a combination of 1 mg L^{-1} NAA or PAA and 1 mg L^{-1} BAP were the optimum conditions for callus induction in *K. alvarezii*. Concentrations of NAA or PAA of 10 mg L^{-1} and higher decreased callus induction. Similar results were also shown by Munoz et al. [78] using 1 mg L^{-1} NAA and BAP, which increased *K. alvarezii* callus induction by 129% compared to the treatment without PGRs (control). Auxins, such as IAA, 2,4-D, and NAA, are known to regulate several physiological processes, including callus formation, cell elongation, and adventitious bud formation. Callus growth can be accelerated by a combination of auxins and cytokinins, such as IAA and BAP, respectively [82]. IAA is a natural auxin that is sensitive to light and is easily degraded; hence, the use of IAA for callus induction must consider light irradiance. Synthetic auxins (IBA, NAA, 2,4-D, and PIC) are more stable to light [68].

Growth stimulants as well as growth retardants, such as UNI and ALA, can be used to induce seaweed calli [44,45,81]. A concentration of 100 mg L^{-1} ALA was optimal in inducing callus formation in all segments of *S. japonica*, but at a concentration of 750 mg L^{-1}, a callus-like cell growth was inhibited. High levels of ALA inhibit growth because of its herbicidal activity. Herbicidal activity tends to increase over cultivation time [44].

In *the Sargassum* genus, supplementation with UNI at concentrations of 5 µM (in *S. horneri*) and 7 µM (in *S. polycystum*) showed the highest callus induction among other PGRs, including IAA, IBA, NAA, PIC, 2,4-D, BAP, KIN, CPPU, and GA [45,81]. UNI is a triazole-type inhibitor of the biosynthesis of cellulose, GA, and brassinosteroids; catabolism of abscisic acid; and the cytochrome P450 enzyme of t-zeatin biosynthesis [83–87]. UNI may inhibit cellulose synthesis in *S. horneri*, leading to callus formation [45].

3.5. The Influence of Gelling Conditions

Several studies on the influence of gelling conditions of media on callus induction in seaweeds have been published [23,40,60,77,88,89]. Data extracted from these articles are presented in Table 5.

Table 5. Studies on the influence of gelling conditions on the callus induction of seaweeds.

Species	Gelling Condition	Result	Reference
Cytoseira osmundacea	0 or 1.5% of agar.	No calli were induced from explants in liquid media, but in 1.5% agar media, explants showed callus induction up to 11%.	[23]
Egregia menziesii	0 or 1.5% of agar.	No calli were induced from explants in liquid media, but in 1.5% agar media, explants showed callus induction up to 9%.	[23]
Enteromorpha intestinalis	0 or 1.5% of agar.	Callus induction rate of 0.07–5% from explants in liquid media, but in 1.5% agar media, explants showed callus induction up to 91%.	[23]
Eucheuma uncinatum	0 or 1.5% of agar.	No calli were induced from explants in liquid media, but in 1.5% agar media, explants showed callus induction up to 3%.	[23]
Gelidium robustum	0 or 1.5% of agar.	No calli were induced from explants in liquid media, but in 1.5% agar media, explants showed callus induction up to 2%.	[23]
Gigartina exasperata	0 or 1.5% of agar.	No calli were induced from explants in liquid media, but in 1.5% agar media, explants showed callus induction up to 3%.	[23]
Gracilaria papenfussii	0 or 1.5% of agar.	No calli were induced from explants in liquid media, but in 1.5% agar media, explants showed callus induction up to 2%.	[23]
Grateloupia doryphora	0.3, 0.8 or 1.5% of agar.	Increasing the agar concentration increased the callus formation, optimal range 0.8–1.5% of agar.	[89]
Hizikia fusiformis	0.1, 0.2, 0.5, 0.7, 1.0, or 1.5% of agar or agarose or alginic acid or high gel strength agar or phytagel or purified agar or transfer agar.	The highest induction rate (47%) occurred with 1% agar and 2% high gel strength agar. All gelling agents showed induction rates $\leq$ 20% at concentrations less than 1%.	[88]
Kappaphycus alvarezii	0.8–3% of agar	Explants at media with 0.8% agar showed a low callus induction rate. The induction rate was high at 1.5% agar (82%), and decreased at 3% agar (64%).	[40]
Kappaphycus alvarezii	0, 0.8, or 1.0% of agar PES medium	Explants induced calluses only on agar 0.8 and 1.0% (20 and 38%, respectively).	[60,77]
Kappaphycus alvarezii	0 or 1.5% of agar.	Explants in liquid media showed a callus induction up to 2%, but in 1.5% agar media, they showed callus induction up to 10%.	[23]
Laminaria sinclairii	0 or 1.5% of agar.	Explants in liquid media induced no calli, but in 1.5% agar media, showed callus induction up to 28%.	[23]
Laurencia sp.	0.3, 0.8, or 1.5% of agar.	The increasing of agar concentration increased the callus formation; optimal at 0.8–1.5% of agar.	[89]
Macrocystis pyrifera	0 or 1.5% of agar.	Explants in liquid media showed callus induction by up to 2%, but in 1.5% agar media, they showed callus induction up to 35%.	[23]
Pelvetia fastigiata	0 or 1.5% of agar.	Explants in liquid media induced no calli, but in 1.5% agar media, they showed callus induction up to 20%.	[23]
Porphyra lanceolata	0 or 1.5% of agar.	Explants in liquid media induced calluses up to 2%, but in 1.5% agar media, they showed callus induction up to 84%.	[23]
Porphyra nereocystis	0 or 1.5% of agar.	Explants in liquid media induced calluses up to 4%, but in 1.5% agar media, they showed callus induction up to 90%.	[23]
Porphyra perforata	0 or 1.5% of agar.	Explants in liquid media induced calluses up to 4%, but in 1.5% agar media, they showed callus induction up to 89%.	[23]
Prionitis lanceolata	0 or 1.5% of agar.	Explants in liquid media induced no calli, but in 1.5% agar media, they showed callus induction up to 1%.	[23]
Sargassum fluitans	0 or 1.5% of agar.	Explants in liquid media induced no calli, but in 1.5% agar media, they showed callus induction up to 15%.	[23]
Sargassum muticum	0 or 1.5% of agar.	Explants in liquid media induced callus up to 0.1%, but in 1.5% agar media, they showed callus induction up to 27%.	[23]
Smithora naiadum	0 or 1.5% of agar.	Explants in liquid media induced callus up to 4%, but in 1.5% agar media, they showed callus induction up to 85%.	[23]
Ulva angusta	0 or 1.5% of agar.	Explants in liquid media induced callus up to 3%, but in 1.5% agar media, they showed callus induction up to 92%.	[23]

Polne-Fuller and Gibor [23] reported that 19 seaweeds, consisting of 2 Chlorophyta, 10 Rhodophyta, and 7 Phaeophyta, developed poorly in liquid media (0% agar). However, all these seaweeds showed callus growth on 1.5% agar media. The calluses were induced on agar or carrageenan media, whereas cells developed erect shoots in liquid media. Considering the high induction rate on solid medium, it is reasonable to presume that the explants implanted for callus induction also require air exposure. Since agar and

carrageenan demonstrated the same callus induction rate, the gelling agent was thought to have no callus-inducing characteristics. Jin et al. [88] studied the influence of various gelling agents at different concentrations on callus induction in *H. fusiformis*. They reported that all gelling agents showed an induction rate of less than 20%, at concentrations of less than 1%. The highest induction rate (47%) was observed with 1% agar and 2% high gel-strength agar.

For *Laurencia* sp. and *Grateloupia doryphora*, an increase in the agar concentration increased callus formation, with the optimal range being 0.8–1.5% agar [89]. An increase in agar content is positively associated with callus development and negatively related to bud regeneration [89]. Rhodophyta *K. alvarezii* showed optimal callus induction in solid media with 1.5% agar, compared to media with 0–1% agar. However, induction decreased when the agar concentration increased to 3% [40,60,77]. This is because the majority of calluses form on solid, but moist, surfaces [23]. The application of axenic tissue culture and the stock maintenance of additional seaweed species will necessitate screening for the most acceptable gelling agents.

3.6. The Influence of Other Factors

Other factors that have been found to influence the rate of callus induction are salinity, osmolality, and water turbulence [44,89,90]. The results of these studies are shown in Table 6.

Table 6. Studies on the influence of salinity, osmolality, and turbulence on callus induction in seaweeds.

Species	Culture Condition	Result	Reference
Laurencia sp.	Different osmolality: 0.5, 0.7, 1.0, or 1.5 Os kg^{-1}	Callus induced at 0.7–1.0 Os kg^{-1}. At 0.5 or 1.5 Os kg^{-1}, callus induction was inhibited or reduced.	[89]
Saccharina japonica	Different salinity: 15, 17.5, 20, 22.5, 25, 27.5, 30, or 35 psu	Callus induced at salinity 25–35 psu (30–60%); optimal at 27.5–30 psu.	[44]
Solieria filiformis	Different forms of turbulence: stationary, air, or water turbulence	Explants in media with air turbulence showed a higher callus induction rate (90.3%), than with water turbulence (4.0%) and stationary (0.0%).	[89]

Salinity and osmolality are physical characteristics of the media that can be easily modulated. Robaina et al. [89] reported the effect of PES media osmolality on callus induction in *Laurencia* sp. Different osmolality conditions (0.5–1.5 Os kg^{-1}) were obtained by diluting seawater with distilled water or by adding sodium chloride. The osmolality levels of 0.7–1.0 Os kg^{-1} were the best for callus induction in *Laurencia* sp.; osmolalities lower or higher than this range decreased the induction rate. The optimal osmolality was found to be similar to that of seawater.

The salinity of the medium also influences callus induction. Calli formation in *S. japonica* was induced under salinities between 25 and 35 psu, but when the salinity was lower than 25 psu, callus formation was not induced. The optimal salinity for callus induction is 27.5–35 psu [44]. The natural environment in which *S. japonica* is distributed has a salinity of 31.3–36.8 psu, while it is farmed in waters with a salinity of 30.0–33.1 psu [91,92]. The natural salinity conditions were in accordance with the optimal conditions obtained for inducing callus formation. The exposure to hyposaline conditions (low salinity) causes the loss of pigment in some Laminariales [93], as well as in *S. japonica*, which results in the discoloration and erosion of the cells [44]. Therefore, both optimal osmolality and salinity of the medium for callus induction could influence the seaweed harvesting site. The results of this study require further investigation, especially in other species.

The Rhodophyta *Solieria filiformis* shows callus induction in liquid medium. The liquid media were treated with three different turbulence systems: stationary, air turbulence, and water turbulence. Air turbulence treatment was generated by air injection through a pipe, whereas water turbulence was generated by water circulation. Hence, the rotary speed in air turbulence is higher than that in water turbulence. Among the three turbulence systems, air turbulence showed the highest callus induction (90.3%), followed by water turbulence (4.0%); the stationary system showed no induction (0%) [90]. Callus induction in seaweeds

by both air- and water-turbulence systems might be a response to abrasion of the apices against the tank walls during water movement. Higher water movement in turbulent air increases the intensity of the shear forces. Therefore, callus induction stimuli generated by movement against the tank walls were also increased. As discussed in Section 3.5, there are only a few seaweeds that show calli induction in liquid conditions. Therefore, the turbulent water treatment used on *S. filiformis* can be applied to these seaweeds. In the future, callus suspension culture should be attempted by cutting the induced calli from solid media and then transferring them to liquid media.

4. Conclusions

This review provides information on the role of abiotic factors in seaweed callus induction. Our investigation revealed that callus induction in seaweeds is influenced by abiotic factors, including light irradiance, temperature of media, media types, plant growth regulators, gelling conditions, osmolality, salinity, and turbulence in liquid media. For callus induction, three important light factors were considered: intensity, period, and spectrum. Most seaweeds showed callus induction in low light intensity in the period of 12–16 h. Further research is needed on various species related to the light spectrum. To achieve optimum callus induction, the temperature, osmolality, and salinity of the media must be controlled. For species that have not been investigated, these criteria can be adjusted based on natural habitat characteristics. Media that can be used for seaweed callus induction are PES and ASP-12-NTA. These media can be modified by supplementing with carbon, vitamins, calcium, or PGRs. The most common PGR treatments for seaweed callus induction are IAA, BAP, 2,4-D, and NAA. The combination of auxins (IAA, IBA, NAA, 2,4-D, and PIC) and cytokinins (BAP, ZEA, KIN, and 2iP) can be considered to improve the induction rate. Most seaweed explants show callus induction on solid media that are exposed to the air-medium interface. Media containing 1.0–1.5% agar are generally preferred for callus induction in seaweed. Turbulence in the liquid medium affects callus induction. Although studies on this topic are limited, it may be feasible to enhance the production of the callus mass in optimal suspension cultures under appropriate abiotic conditions, including turbulent water. Further studies are needed to establish the optimal conditions for sustaining or promoting large-scale callus production in suspension cultures for seedling and metabolite production.

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