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Micropropagation as a tool for sustainable utilization and conservation of populations of Rhodophyta

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Abstract: Micropropagation as a tool for sustainable utilization and conservation of populations of Rhodophyta. Micropropagation, or *in vitro* clonal propagation, allows the production of a large number of individuals within a short period. These micropropagated clones could be used as seedlings for seaweed cultivation, avoiding collection from natural beds. Consequently, there has been an increasing interest in micropropagation as a tool for preservation of populations of marine red algae on the Brazilian coast and for the sustainable production of raw material for commercial exploration. This paper reviews the literature on tissue culture and micropropagation of red algae published during the three last decades. Based on the literature, we can conclude that the regeneration process is complex and diversified in different species of Rhodophyta and that the success of micropropagation depends on this process. Species belonging to the orders Bangiales and Ceramiales showed low potential for regeneration, while Gigartinales species showed the highest potential for regeneration. Micropropagation of commercially important red algae is fundamental for the conservation of natural populations by providing seedlings for cultivation and for germplasm collections, both for the conservation of genetic diversity and for biotechnological applications.

Introduction

Seaweed tissue culture is an important tool for micropropagation, as well as for the selection of high-yield strains or those with interesting commercial characteristics. Micropropagation, or *in vitro* clonal propagation, permits the production of a large number of individuals within a short period. These can then be used as seedlings for seaweed cultivation, rather than collecting them from natural beds. This had generated an increasing interest in micropropagation as a tool for the preservation of populations of marine red algae and for the sustainable production of raw materials of commercial interest (Scheme 1).

Micropropagation is based on cell totipotency, which is the capacity of a single vegetative cell to divide and differentiate to generate a new organism. This phenomenon is the basis of plant tissue culture and is used to analyze the physiological and biochemical processes of plant differentiation and development.

Tissue culture (or *in vitro* culture) is defined as the axenic culture of any part of one organism (called an explant) under controlled conditions in a culture medium. Seaweed tissue culture differs from unialgal culture in relation to the axenic condition (without bacteria and fungi) and some of the characteristics of the culture medium used for the tissue culture (Yokoya, 1996). Although the thallus of seaweed does not differentiate into tissue, the term "tissue culture" is used to refer to the axenic conditions of seaweed culture using different explants (thallus segments, cortical and/or medullar regions, filaments, and callus).

The first attempt to cultivate seaweed explants under axenic conditions was performed by Aharon Gibor in 1950 (Polne-Fuller, 1988). However, the procedures used for plant tissue culture were not suitable for seaweeds due to two main factors: the difficulties in obtaining axenic algal explants and the poor knowledge of the physiological processes involved in seaweed development, including the role played by plant growth regulators (Polne-Fuller, 1988). In 1978, Chen & Taylor described the axenic culture of the medullar region of *Chondrus crispus* Stackhouse (Rhodophyta), which could be considered the first successful seaweed tissue culture. Following their publication, several studies were carried out in order to develop methods for obtaining axenic explants and to investigate the factors that affect seaweed tissue culture. For successful seaweed tissue

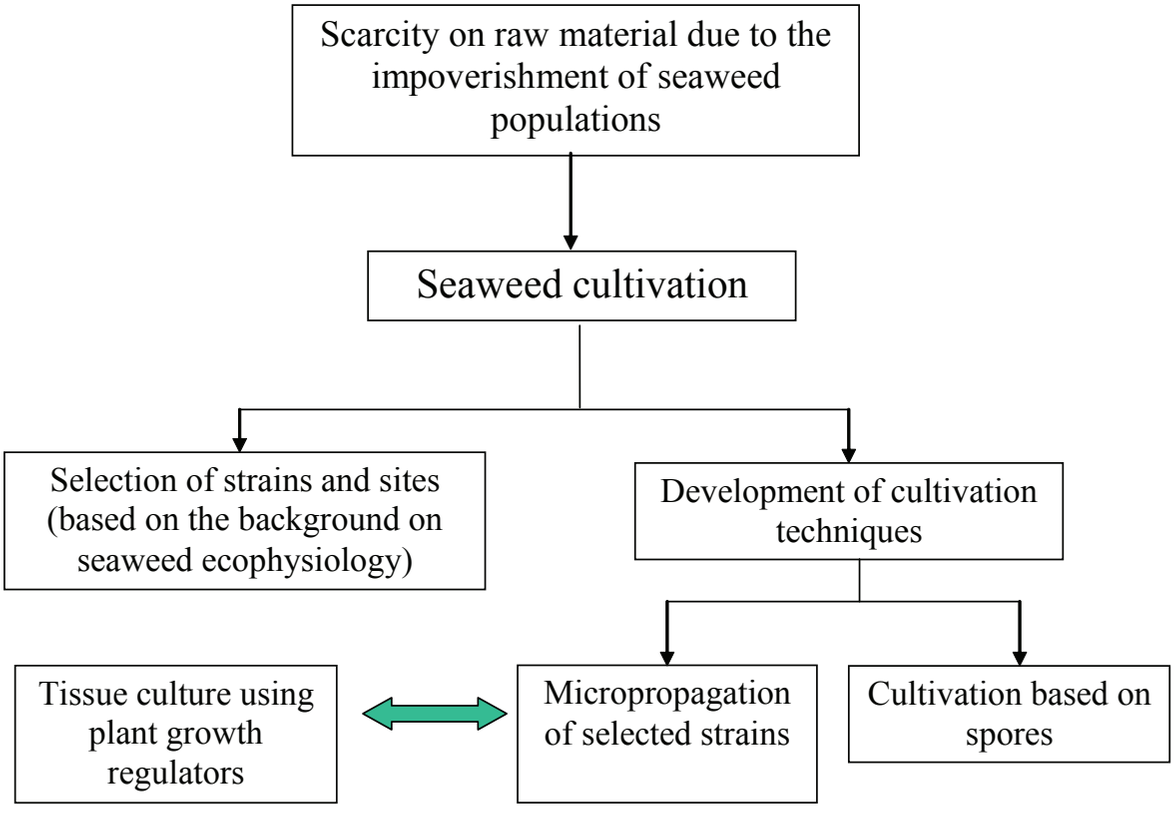
culture, it is of fundamental importance to know the physiology of seaweed and the factors that control seaweed development. These factors can be divided into two groups: endogenous factors (related to explant characteristics such as origin, physiological state, stage of life history, age etc) and exogenous factors (such as temperature, salinity, irradiance, photoperiod, chemical composition and pH of the culture medium, state of medium (liquid or solid), addition of plant growth regulators and/or inorganic carbon to the culture medium).

Plant growth regulators (PGR) in Rhodophyta

The main objective of the first publications on seaweed tissue culture was to investigate callus induction and growth in different species of red algae (Table 1). Polne-Fuller & Gibor (1987) described callus formation in nine species of rhodophytes, including species belonging to the genera *Porphyra, Gelidium, Gracilaria, Eucheuma, Gigartina* and *Prionitis*, but regeneration from callus was not observed. Similar results were reported by Gusev et al. (1987) for species of *Ceramium, Laurencia, Gracilaria, Furcellaria* and *Phyllophora* and also by Liu & Gordon (1987) for *Pterocladiella capillacea*. Other studies reported similar results for *Porphyra umbilicalis* (Liu & Kloareg, 1991), *Grateloupia doryphora* (Robaina et al., 1990), *Eucheuma denticulatum* (Dawes & Koch, 1991), *Laurencia* sp. (Robaina et al., 1992), *Gracilaria verrucosa* (Kaczyna & Megnet, 1993) and *Solieria filiformis* (Robledo & Garcia-Reina, 1993).

Regeneration from callus was reported for *Agardhiella subulata* (Bradley & Cheney, 1990) and *Grateloupia filiformis* (Yokoya et al., 1993), which belong to the order Gigartinales. Huang & Fujita (1996) studied ten species of Gigartinales and, among these, six species showed potential for regeneration from callus, notably the genera *Carpopeltis, Grateloupia* and *Prionitis*. Species of *Grateloupia* showed the highest regeneration potential, which could be increased by the addition of plant growth regulators (auxins and/or cytokinins), as reported for *Grateloupia dichotoma* by Yokoya & Handro (1996, 1997). For other species of Gigartinales, regeneration from callus was found to be stimulated by PGR in *Solieria filiformis* (Yokoya & Handro, 2002), *Hypnea musciformis* (Yokoya et al., 2003) and *Kappaphycus alvarezii* (Muñoz et al., 2006). However, the presence of PGR was facultative for the last two species, as reported by Bravin et al. (2006) and Reddy et al. (2003), respectively.

In contrast to Gigartinales species, in species belonging to Gracilariales the PGR play an important role in inducing the regeneration process, stimulating



Scheme 1. Flow diagram of the steps involved in the utilization of micropropagation as a tool for the conservation of seaweed populations and for sustainable production of raw material of commercial interest.

cell differentiation in the callus of *Gracilaria vermiculophylla* (Yokoya et al., 1999), *G. chilensis* (Collantes et al., 2004), *G. domingensis* (Ramlov, 2007), *G. perplexa* and *G. tenuistipitata* (Yokoya et al., 2004), and *Gracilariopsis tenuifrons* (Yokoya, 2000).

Based on studies published during the last three decades, we can conclude that the regeneration from callus is complex and diversified in the different species of Rhodophyta and that the success on micropropagation will ultimately depend on this process. The data indicate the need for further investigation into the role played by PGR on cell processes (division, elongation, and differentiation) in the different species, considering the potential for regeneration of each order. Species belonging to the orders Bangiales and Ceramiales showed low potential for regeneration from callus, while Gigartinales species showed the highest potential (Table 1).

Biotechnological applications

Few studies have reported the use of micropropagation for biotechnological applications and improvement in this field needs to be achieved. Maliakal et al. (2001) reported the production of halogenated compounds from adventitious plantlets regenerated from callus of *Ochtodes secundiramea*, and Reddy et al. (2003) selected specimens originated from micropropagules regenerated from callus of *Kappaphycus alvarezii* that had higher growth rates than specimens farmed in India.

Table 1. Effects of plant growth regulators (PGR, auxins and cytokinins) on callus formation and thallus regeneration from callus in some species of Rhodophyta. Modified from Yokoya (2007).

Species	Callus	Regeneration from callus		References
	PGR		PGR	
BANGIALES				
Porphyra lanceolata (Setchell & Hus) G.M. Smith	N	-	NT	Polne-Fuller & Gibor (1987)
Porphyra nereocystis C.L. Anderson	N	-	NT	Polne-Fuller & Gibor (1987)
Porphyra perforata J. Agardh	N	-	NT	Polne-Fuller & Gibor (1987)
Porphyra umbilicalis (L.) Kuetzing	ST	-	NT	Liu & Kloareg (1991)
Smithora naiadum (C.L. Anderson) G.J. Hollenberg	N	-	NT	Polne-Fuller & Gibor (1987)
CERAMIALES				
Ceramium kondoi Yendo	ST	-	NT	Gusev et al. (1987)
Laurencia paniculata Kuetzing	ST	-	NT	Gusev et al. (1987)
Laurencia sp.	NT	-	NT	Robaina et al. (1992)
Laurencia undulata Yamada	ES	-	NT	Huang & Fujita (1996)
GELIDIALES				
Gelidiella acerosa (Forsskål) Feldmann & G. Hamel	N	+	N	Kumar et al. (2004)
Gelidium nudifrons N.L. Gardner	N	-	NT	Polne-Fuller & Gibor (1987)
Gelidium robustum (N.L. Gardner) Hollenberg & I.A. Abbott	N	-	NT	Polne-Fuller & Gibor (1987)
Gelidium vagum Okamura	ST	-	NT	Gusev et al. (1987)
Gelidium versicolor (Gmel.) Lamour.	NT	+	NT	Garcia-Reina et al. (1988)
Pterocladiella capillacea (S.G. Gmelin) Santelices & Hommersand (as *Pterocladia capillacea*)	ES	-	NT	Liu & Gordon (1987)
Ptilophora subcostata (Okamura) N.E. Norris	ST	-	NT	Huang & Fujita (1996)
GRACILARIALES				
Gracilaria chilensis Bird, McLachlan & Oliveira	NT	+	NT	Collantes et al. (2004)
Gracilaria domingensis (Kutzing) Sonder ex Dickie	ST	+	ST	Ramlov (2007), Ramlov et al. (2009)
Gracilaria papenfussi I.A. Abbott	N	-	NT	Polne-Fuller & Gibor (1987)
Gracilaria perplexa Byrne & Zuccarello	ST	+	ST	Yokoya et al. (2004)
Gracilaria tenuistipitata Chang & Xia	ST	+	ST	Yokoya et al. (2004)
Gracilaria textorii Hariot	N	-	NT	Huang & Fujita (1996)
Gracilaria verrucosa (Hudson) Papenfuss	ST	-	NT	Gusev et al. (1987); Kaczyna & Megnet (1993)

Gracilaria vermiculophylla (Ohmi) Papenfuss	ST	+	ST	Yokoya et al. (1999)
Gracilariopsis tenuifrons (Bird & Oliveira) Fredericq & Hommersand	ES	+	ST	Yokoya (2000)
RHODYMENIALES				
Rhodymenia pertusa (Postels & Ruprecht) J. Agardh	ST	-	NT	Gusev et al. 1987
GIGARTINALES				
Agardhiella subulata (C. Agardh) Kraft	ES	+	ST	Bradley & Cheney (1990)
Ahnfeltiopsis flabelliformis (Harvey) Masuda	ST	-	NT	Huang & Fujita (1996)
Carpopeltis affinis (Harvey) Okamura	ST	+	NT	Huang & Fujita (1996)
Carpopeltis prolifera Kawaguchi et Masuda	ST	+	NT	Huang & Fujita (1996)
Chondracanthus tenellus (Harvey) Hommersand	ST	-	NT	Huang & Fujita (1996)
Eucheuma denticulatum (N.L. Burman) F.S. Collins & Hervey	ES	-	NT	Dawes & Koch (1991)
Eucheuma uncinatum Setchell & Gardner	N	-	NT	Polne-Fuller & Gibor (1987)
Furcellaria fastigiata (Turner) J.V. Lamour.	ST	-	NT	Gusev et al. (1987)
Gigartina exasperate Harvey & Barley	N	-	NT	Polne-Fuller & Gibor (1987)
Gloiopeltis tenax (Turner) Decaisne	ST	-	NT	Huang & Fujita (1996)
Grateloupia acuminata Holmes	ST	+	NT	Huang & Fujita (1996)
Grateloupia dichotoma J. Agardh	ST	+	ST	Yokoya & Handro (1996; 1997)
Grateloupia doryphora (Montagne) M.A. Howe	NT	-	NT	Robaina et al. (1990)
Grateloupia filicina C. Agardh	ST	+	NT	Huang & Fujita (1996)
Grateloupia filiformis Kuetzing	NT	+	NT	Yokoya et al. (1993)
Grateloupia imbricata Holmes	ST	+	NT	Huang & Fujita (1996)
Grateloupia turuturu Yamada	ST	+	NT	Huang & Fujita (1996)
Hypnea musciformis (Wulfen in Jacqu.) J.V. Lamour.	ST NT	+ -	ST NT	Yokoya et al. (2003); Bravin et al. (2006)
Kappaphycus alvarezii (Doty) Doty ex P.C. Silva *Kappaphycus alvarezii*	N ST	+ +	N ST	Reddy et al. (2003); Muñoz et al. (2006); Hayashi et al. (2008)
Meristotheca papulosa J. Agardh	ST	+	NT	Huang & Fujita (1997)
Ochtodes secundiramea (Montagne) M.A. Howe	NT	+	NT	Maliakal et al. (2001)
Phyllophora nervosa (A.P. de Candolle) Greville	ST	-	NT	Gusev et al. (1987)
Prionitis crispata Kawaguchi	ST	+	NT	Huang & Fujita (1996)
Prionitis lanceolata (Harvey) Harvey	N	-	NT	Polne-Fuller & Gibor (1987)
Solieria filiformis (Kuetzing) Gabrielson	NT	-	NT	Robledo & Garcia-Reina (1993)
Solieria filiformis	N	+	ST	Yokoya & Handro (2002)

ST: stimulatory effect; ES: plant hormones (auxins and cytokinins) are essential and their effects are stimulatory; N: without effect; NT: not tested; -: absence of thallus regeneration; +: presence of thallus regeneration.

Conclusions

Production and selection of new seaweed strains could improve the synthesis of natural products with different biological activities, as well as the commercial cultivation of seaweeds via the utilization of higher yield strains. Moreover, micropropagation of the commercially important red algae is fundamental for the conservation of natural populations, providing seedlings for cultivation and for germplasm collections. These seedlings could be used both for the preservation of genetic diversity and in biotechnological applications.

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