REGENERATION OF THALLICLONES[1] FROM *LAURENCIA* SP. (RHODOPHYTA)

Guillermo Garcia-Reina, Rafael R. Romero and Angel Luque

Universidad Politecnica Canarias, Departamento de Biologia, Box 550, Las Palmas de Gran Canaria, Spain

INTRODUCTION

Tissue culture techniques could be applied to improve the genetic qualities of seaweeds. In order to apply those techniques, efficient methods for obtaining and regenerating calli are needed. The term "callus" or "calluslike" has been applied to define very different structures in seaweeds (Dixon 1963; Fries 1980; Chen 1982; Saga et al. 1982; Tsekos 1982; Polne-Fuller et al. 1984; Yan 1984). Histological studies have been performed on "tumour-like" growths induced by bacteria in *Gigartina teedii* (Tsekos 1982), and to our knowledge nothing is known on the cytological events preceding differentiation from true callus. In a previous paper (Garcia-Reina et al. 1987), we reported the spontaneous formation of morphogenetic calli, the different calligenic potentials among *Laurencia* species and primary explants, and the lack of necessity for axenicity. Callus growth was drastically reduced after its organogenetic trigger, and the organogenetic potential seemed to decrease with time in culture.

The objective of this paper was: (1) to study the effect of different physical states of the culture media and its supplementation with several hormones to determine their effect on *Laurencia* callus induction, growth and in vitro regeneration, and (2) the histological study of the callus, in an attempt to clarify the callus versus calluslike composition.

---

[1] Thalliclones = thalli regenerated from callus.

MATERIAL AND METHODS

Apical branched segments (5 mm long) from Laurencia sp. were used as the primary explant. Two hours after collection they were subjected to the "sonication - 1% Betadine - GAN" treatment described previously (Garcia-Rheina et al. 1987) and cultured in petri dishes with 20 ml Provasoli-enriched seawater media (PES) (Provasoli 1968). Seawater (not the enrichment) was diluted 70 and 50% with distilled water in the "seawater assay". PES was supplemented with GEO_2 (0.5 mg l^{-1}), Ampicillin (10 mg l^{-1}), Nyastatin (2 mg l^{-1}), (GAN) and agar (Bacto Difco, 8 g l^{-1} agar were tested; also 45 explants were cultured in PES ± GAN liquid media. Two or 5 mg l^{-1} kinetin, BA or 2,4-D was added to the media in the "hormone assay". The effect of 5 mg l^{-1} Na-Naphtenate (Pfalz and Bauer, Inc., NO0910) was also tested.

The cultures were incubated in a growth chamber adjusted to 21 + 2°C, 18 h light and 1500 lx. Transfers of cultures to fresh media were done at 15-day intervals. After 45, 60, 75 and 90 days the number of buds arising from the calli were evaluated. Calli with a homogeneous phenotype were transferred to an aerated liquid medium (PES + GAN) after 60 days in culture. The histological study of calli was performed following previously described methods (Devilopoulos and Tsekos 1986). Semi-thin sections (0.5 µ) were stained with toluidine blue and post-stained with lugol.

RESULTS

After 45 days in culture (uni-algal, non-axenic), we found different types of growth: (1) callus arising from the cut surface or from the disorganization of the apical neogrowth, with or without thalliclonal regeneration; (2) finger like growths of buds from the cut or apical zone; (3) thallus arising from the cut end or developing from the buds of the primary explant.

Reducing seawater concentration (osmotic potential) to 70 and 50% halted development of any type of growth (Table 1)

Reducing agar concentration (matric potential) produced an increase in the number of viable explants, but a decrease in the number of explants producing callus (Table 1) None of the explants in liquid media produced callus.

Table 1. Effects of the different assays on the type of growth after 45 days in culture

Assay[b]		N	Type of growth[a] V	C	F	T
Seawater	50%	45	0	-	-	-
	70%	45	2	0	0	2
	100%(*)	45	16	10	2	4
Agar (g l^{-1})	3	45	43	0	0	43
	8 (*)	45	17	7	4	6
	20	45	17	17	0	0
2 mg l^{-1} Kinetin		30	21	15	4	2
2 mg l^{-1} BA		30	17	8	6	3
2 mg l^{-1} 2,4-D		30	28	18	7	3
5 mg l^{-1} Kinetine		30	0	-	-	-
5 mg l^{-1} BA		30	0	-	-	-
5 mg l^{-1} 2,4-D		30	0	-	-	-
5 mg l^{-1} Na-Naph.		30	0	-	-	-
Control (*)		30	11	7	0	4

[a]V= Viability (number of explants showing any type of growth after 45 days), C= callus, F= finger, T= tallus. [b](*)= control = PES + GAN + 8 g l^{-1} agar.

Two mg l^{-1} kinetin, BA or 2,4-D increases viability and number of explants forming callus. Five mg l^{-1} kinetin, BA, 2,4-D or Na-Naphtenate has an inhibitory effect upon any type of growth (Table 1). Morphogenetic calli have an average of three to four buds after 45 days. No differences in callus growth and number of regenerating buds were observed among agar assay, hormone assay and the control. Differences were among individual calli. Later controls (60, 75 days) of the highest morphogenetic calli (at 45 days) show a decrease in the number of buds. The decrease is related to the fusion of thalliclones. Calli can be cultured for more than 5 months in agarized media, thus retaining their morphogenetic potential, and producing "air-growing" thalliclones.

In liquid media some calli developed many air-growing thalliclones around the callus, while others developed a few slow-growing thalliclones, despite showing a homogeneous phenotype

at the transfer. Fast- and slow-growing lines have retained their phenotype for at least 4 months in culture.

Histological study under light microscopy shows a clear difference between the cells from the explant and the callus. Callus cells are small, meristematic and filled with florid granules, which became brown-black after being post-stained with lugol. Enlarged cells and wide intercellular spaces seem to be oriented to the regenerating areas on the surface of the calli. Toluidine blue stained the calli metachromatic.

DISCUSSION

The physical state of the media (agarized) seems to be a key factor in the induction of callus in seaweed (Fries 1980; Chen 1982; Saga et al. 1982; Saga and Sakai 1983; Neushul 1984; Polne-Fuller et al. 1984; Lee 1985). Our results show that the decrease of the hydric potential (more negative) enhances callus formation related to the matric and osmotic potentials (percent agar and seawater respectively). However, Polne et al. (Polne-Fuller et al. 1984) reported callus formation in several species of Porphyra in PES supplemented with 0.3% agar, and callus from protoplasts of several green species when grown on 1% agar, while reporting only thalli when grown on 0.5% agar (Polne-Fuller et al. 1986). Differences between species (and their habitats) may be involved.

The effect of the addition of hormones to seaweed media in order to obtain or regenerate callus is confusing with regards to the effects and type of hormones and concentrations (Bradley and Cheney 1986; Chen 1982). Na-Naphtenate (5 mg l^{-1}) has been described as a "crucial hormone" for inducing callus in Laminaria japonica (Yan 1984). Our results suggest that great differences in the in vitro uptake, metabolism and effect of plant hormones may exist between seaweed species.

The high facility of the buds arising from the calli cultured in agarized media to us together, and of the apex of the thalliclones to differentiate into a disc, indicates the high platicity of the apex of Laurencia sp. The fusion among regenerated buds shows that no loss of the morphogenetic potential, nor a mechanism of dominance, is involved in the apparent mor-

phogenetic potential decline. High plasticity and ano dominance seem to be desirable characteristics for selecting a primary explant to start tissue cultures.

The spontaneous apparition of fast- and slow-growing lines and its apparent stability indicate the usefulness of seaweed tissue culture for selecting superior strains.

The dimensions and high concentration of starch grains in callus cells resemble apical cells and carpospores (Devilopoulos and Tsekos 1986) of tip regions of Laurencia and other tubular branched Rhodophyta. Thalliclones show the normal histologly of Laurencia thalli. Copious deposition of starch in plastids has been described as the first visible change accompanying callus differentiation of the moss Psicomitrium coorgense (Lal and Narang 1985). Laurencia calli are cell systems closely related to the calli of higher plants, but different from structures referred to as calli in brown seaweeds.

REFERENCES

Bradley PM and Cheney D (1985) Stimulation of cell division in a marine red alga using plant growth regulating substances. Plant Physiol Suppl 77:69

Chen LCM (1982) Callus-like formations from Irish moss. Biol Bull Nat Taiwan Nor Univ 17:63-68

Devilopoulos SG and Tsekos I (1986) Ultrastructure of carposporogenesis in the red alga Gracilaria verrucosa. Bot Mar 29:27-35

Dixon PG (1963) Biology of the Rhodophyta. Oliver & Boyd, Edinburgh

Fries L (1980) Axenic tissue cultures from the sporophytes of Laminaria digitata and Laminaria hyperborea. J Phycol 16: 475-477

Garcia-Reina G, Romero RR and Brinkhuis B (1987) Induction of morphogenetic callus from Laurencia (Rhodophyta). J Phycol (submitted)

Lal M and Narang M (1985) Ultrastructural and histochemical studies of transfer cells in the callus and apogamous sporophytes of Psicomitrium coorgense Broth. New Phytol 100:225-231

Lee TF (1985) Aposporous gametophyte formation in stipe explants from Laminaria saccharina. Bot Mar 28:179-185

Neushul M (1984) Marine biomass program plant breeding and genetics. Gas Res Inst Rep N. 84/0076

Polne-Fuller M, Biniaminov M and Gibor A (1984) Vegetative propagation of Phorphyra perforata. Hydrobiologia 116/117:308-313

Polne-Fuller M, Saga N and Gibor A (1986) Algal cell, callus and tissue cultures and selection of algal strains. Beihefte zur Nova Hedwigia 83:30-36

Provasoli L (1968) Media and prospects for the cultivation of marine algae. In Watanabe A, Hatori A (eds) Cultures and col lection of marine algae. Jap Soc Plant Physiol, p 3075

Saga N, Motomura T and Sakai Y (1982) Induction of callus from the marine brown alga Dyctiosiphon foeniculaceus. Plant and Cell Physiol 23:727-730

Saga N and Sakai Y (1983) Axenic tissue culture and callus formation of the marine brown alga Laminaria angustata. Bull Jap Soc Sci Fish 49:1561-1563

Tsekos I (1982) Tumour-like growths induced by bacteria in the thallus of a red alga, Gigartina teedii. Ann Bot 49:123-126

Yan ZM (1984) Studies on tissue culture of Laminaria japonica and Undaria pinnatifida. Hydrobiologia 116/117:314-316