In vitro plantlet formation and multiple shoot induction in *Sargassum heterophyllum*

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Plantlets of *Sargassum heterophyllum* (Turn.) J. Agardh. were established in aseptic culture in the defined medium ASP-12 NTA. The growth and development of plantlets in this medium was favourable in comparison to that in Provasoli enriched sea water medium and in Von Stosch's medium. Holdfast explants exhibit greater wound healing and regenerative growth than explants from the primary lateral and apical regions.

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Plantjies van *Sargassum heterophyllum* (Turn.) J. Agardh. is deur aseptiese kultuur in die beskrewe medium ASP-12 NTA gevestig. Groei en ontwikkeling het gunstig vergelyk met die in 'n Provasoli-verrykte seewatermedium en in die Von Stosch-medium. Eksplante van 'ankergedeeltes' het meer wondheling en regeneratiewe groei getoon as die van die primêre en apikale gedeeltes.

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**Introduction**

Although the use of tissue culture is well established in higher plant orders, studies utilizing this technique for algal physiology and morphogenesis have been limited (Nakahara & Nakamura 1973; Saga et al. 1978; Fries 1980). Moreover, such studies were conducted with isolated cells or callus-like tissues obtained spontaneously. Progress in the study of algal morphogenesis and physiology therefore depends largely on the use of existing culture methods and their successful modification.

In only a limited number of marine macroalgae have there been attempts to establish axenic cultures in defined media. In most cases growth of the alga concerned was relatively poor. This has been attributed to a necessary mutualistic interaction between the alga and associated micro-organisms, in much the same way that De Wit (1960) recorded for higher plants. Poor algal growth could, however, as easily be attributed to a number of factors related to culture conditions, especially the media used. Although multicellular species have been cultured successfully in some media most of them were developed for unicellular algae. Thus a deficiency in the culture medium might be directly responsible for the poor algal growth frequently reported.

The intentions of this investigation were to establish plantlets of *Sargassum heterophyllum* in aseptic culture in a defined medium, viz. ASP-12 NTA, and to compare the growth of the plantlets in this defined medium with that in two popular, undefined media.

**Materials and Methods**

All material used in this study was collected from shallow rock pools high up in the littoral zone at Rocky Bay on the Natal South Coast, during January 1983. Segments of thalli were excised from different portions of both immature and mature plants using a sharp, sterile scalpel on a glass petri dish. The plant segments were placed into an antibacterial medium for 48 h. The antibacterial medium employed was that described by Guillard (1968), viz. 124.5 mg penicillin G, 50 mg streptomycin sulphate and 20 mg chloramphenicol dissolved in 1 dm³ charcoal-deactivated sea water.

**Organogenesis of immature *Sargassum heterophyllum* explants**

From a total of 30 immature plants, segments of a thallus 10–15 mm in length, were prepared from the apex (including the apical meristem), the primary lateral axis (all 'leaves' and secondary laterals being removed) and the holdfast (including a small portion of the main axis).
Organogenesis of mature *Sargassum heterophyllum* explants

From a total of 30 mature plants, segments of a thallus 10–15 mm in length were cut from the apex (including the apical meristems), the receptacles, the primary lateral axis and the holdfast (including a small portion of the main axis).

After sterilization in the antibiotic mixture for 8 h, the explants were rinsed four times in sterile charcoal-deactivated sea water. The cut ends of the explants were then trimmed aseptically, after which they were placed into 150 mm³ sterile culture medium according to the procedure outlined by Saga & Sakai (1982). The three culture media used throughout were: ASP-12 NTA (Provasoli 1963), Provasoli’s enriched sea water medium (Provasoli et al. 1957) and Von Stosch’s medium (Von Stosch 1963).

The cultures were incubated at 15 °C in a 16 h light 8 h dark, light cycle. Illumination was supplied by cool white fluorescent lamps at 100 μE m² sec⁻¹. The cultures were transferred to new media at monthly intervals.

**Results**

Organogenesis of immature *Sargassum heterophyllum* explants

Table 1 shows the different growth responses of the explants in the three different culture media over the experimental period of 24 weeks. From these results it can be seen that plantlet formation was more marked from the holdfast explants than from the apical meristem and primary lateral axis explants. Holdfast explants grown in Von Stosch’s medium and ASP-12 NTA medium yielded the greatest number of proliferations. Examination of the apical segments from all plants revealed no obvious proliferation of new adventitious shoots from the cut surfaces. Shoot proliferation from the cut surfaces of the primary lateral axis explants was observed only from segments grown in Von Stosch’s medium. Unfortunately, few of these explants survived the entire experimental period.

**Table 1** Number of proliferations produced on *Sargassum heterophyllum* explants from immature and mature tissue after 24 weeks in culture

| Position of segment | Culture medium |
|---------------------|----------------|
|                     | Von Stosch     | Provasoli’s enrichment | ASP-12 NTA |
| Immature explants   |               |                        |            |
| Apex                | –             | –                       | –          |
| Primary lateral     | 3 (5)         | 47 (4)                  | 126 (7)    |
| Holdfast            | 143 (7)       | –                       | –          |
| Mature explants     |               | –                       | –          |
| Apex                | –             | –                       | –          |
| Primary lateral     | –             | –                       | –          |
| Receptacle          | –             | –                       | –          |
| Holdfast            | 54 (7)        | 12 (7)                  | 16 (5)     |

Figures in brackets indicate the number of explants which responded to the different treatments.

Organogenesis of mature *Sargassum heterophyllum*

Examination of Table 1 shows that after the 24-week culture period similar trends in shoot proliferation were observed for the mature and immature plant tissues. No regenerative tissue developed on the cut surfaces of the apical, receptacular or the primary lateral axis explants from mature tissue. The holdfast explants displayed shoot proliferation. The greatest number of proliferations were again observed on holdfast explants grown in Von Stosch’s and ASP-12 NTA media, respectively. Figures 1 & 2 illustrate the proliferation of multiple shoots from the cut surfaces of mature plant holdfast segments.

**Discussion**

The establishment of plantlets of *Sargassum heterophyllum* in aseptic culture in the defined medium ASP-12 NTA was achieved following the procedure outlined by Saga & Sakai (1982). Growth of plantlets in this medium was found to be relatively good in comparison to growth of explants maintained in Provasoli’s enriched sea water medium. It was not, however, as marked as the plantlet formation observed from explants maintained in Von Stosch’s medium.

Both regeneration studies clearly demonstrated that holdfast regions were more active in wound healing and regenerative growth than were middle and apical regions. At the time of completion of the experiment, no new adventitious branches could be seen on the cut surfaces of the apical region segments. These observations suggest that the apical cell region exerts an inhibitory effect on this regenerative process. Similar apical dominance phenomena have been described by Moss (1963, 1964, 1966) in segmented thalli of *Fucus vesiculosus* L. and by Fletcher & Fletcher (1975) in *Sargassum muticum* (Yendo) Fensholt. The latter authors suggested that growth inhibitory hormones are produced at the apical region and are subsequently transported down the axis, the concentration being less near the base of the plants, thus allowing segments cut from this region to develop adventitious branches more readily. There is however, at present, no evidence to support this theory.

Although holdfast segments from both immature and mature plants exhibited marked multiple shoot initiation, the growth and development of the shoots was markedly different. The immature plant holdfast explants developed numerous ‘finger-like’ proliferations from the cut surfaces of the holdfast and a single shoot from the stump of the main axis. The shoot arising from the main axis appeared to exert an apical dominance effect over the numerous proliferations arising from the holdfast surface. In the mature tissue explants, all the shoots developed into plantlets and no single shoot appeared to exert a dominance effect over another.

On the basis of the gross number of proliferations arising from the holdfast, the development of explants of mature *S. heterophyllum* tissue was not as vigorous as that of the immature tissue explants. However, shoot development from mature explants was greater than that from immature explants, since the ‘finger-like’ proliferations produced from the latter explants do not fit the definition of a shoot. A shoot is defined in this instance as being a single structural unit which consists of a stem or axis bearing leaves which ordinarily grow to a standard size and then stop (Simon et al. 1968; Cutter 1978). The development of a shoot from an explant may arise either from meristems present within the explant tissue or from meristemoids developed from a callus produced by the explant. According to Bunning (1952), polarity is necessary for differentiation. Suppression of polarity (by neutralization of internal gradients of nutrients and growth regulators) brings about callusing. Whether the ‘finger-like’ proliferations arising from the immature explants represent undifferentiated tissue is as yet unknown. They did not, however, represent morphologically definable shoots by the end of the experiment. Further anatomical studies would be required to
determine the nature of this tissue. The possibility of these being undifferentiated cells offers enormous potential for future research and perhaps for the eventual establishment of a system whereby the characterization of biosynthesis and metabolism of plant growth regulators in *S. heterophyllum* could be elucidated.

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References

BÜNNING, E. 1952. Morphogenesis in plants. *Surv. Biol. Prog.* 2: 105–138.

CUTTER, E.G. 1978. Plant Anatomy: Experiment and Interpretation. II. Organs. Edward Arnold, London.

DE WIT, C.T. 1960. On competition. *Versl. Landbouwk. Onderz. Ned.* 66: 1–82.

FLETCHER, R.L. & FLETCHER, S.M. 1975. Studies on the recently introduced brown alga *Sargassum muticum* (Yendo) Fensholt. II. Regenerative ability. *Botanica mar.* 18: 157–162.

FRIES, L. 1980. Axenic tissue culture from the sporophytes of *Laminaria digitata* and *Laminaria hyperborea* (Phaeophyta). *J. Phycol.* 16: 475–477.

GUILLARD, R.R.L. 1968. A simplified antibiotic treatment for obtaining axenic cultures of marine phytoplankton. Mimeographed document. *Woods Hole Oceano. Instr.*, Marine Biological Laboratory: 9.

MOSS, B.M. 1963. Wound healing and regeneration in *Fucus vesiculosus*. *Proc. Int. Seaweed Symp.* 4: 117–122.

MOSS, B.M. 1964. Growth and regeneration of *Fucus vesiculosus* in culture. *Br. phycol. Bull.* 2: 377–380.

MOSS, B.M. 1966. Polarity and apical dominance in *Fucus vesiculosus*. *Br. phycol. Bull.* 3: 31–35.

NAKAHARA, H. & NAKAMURA, Y. 1973. Parthenogenesis, apogamy and apospory in *Alaria crassifolia* (Laminariales). *Mar. Biol.* 18: 327–332.

PROVASOLI, L. 1963. Growing marine seaweeds. *Proc. Int. Seaweed Symp.* 4: 9–17.

PROVASOLI, L., McLACHLAN, J.J.A. & DROOP, M.R. 1957. The development of artificial media for marine algae. *Arch. Mikrobiol.* 25: 392–428.

SAGA, N. & SAKAI, Y. 1982. A new method for pure culture of macroscopic algae, the one step selection method. *Jap. J. Phycol.* 30: 40–43.

SAGA, N., UCHIDA, T. & SAKAI, Y. 1978. Clone *Laminaria* from a single isolated cell. *Bull. Jap. Soc. Scient. Fish.* 44: 87.

SIMON, E.W., DORMER, K.J. & HARTSHORNE, J.N. 1968. Lawson's textbook of Botany. University Tutorial Press Ltd., London.

VON STOSCH, H.A. 1963. Wirkung von Jod und Arsenit auf Meeresalgen in Kultur. *Proc. Int. Seaweed Symp.* 4: 142–150.