

REGENERATIVE CAPACITY IN TWO SPECIES OF *HYPNEA*

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The tendrils of excised pieces of *Hypnea musciformis* showed an increase of 10.10% in fresh weight over the initial weight after 45 days of growth. "Stellate" bulbils of *Hypnea valentiae* in another set of experiments showed a total increase of 219.7 cm in length over the original length of 4.3 cm in 60 days of growth. This suggests an easy method of vegetative propagation of these two species for large scale cultivation.

Key words: *Hypnea musciformis*, *Hypnea valentiae*, tendrils, stellate bulbils, regeneration.

Members of Grigartinales, show regeneration from the cut ends of their thallus. Jenkin & MaCombs (1967) have shown that the tendrillar portion of *Hypnea musciformis* contained gibberellin that induce regeneration from the detached tendrills. Similarly, Lipkin (1977) demonstrated in *Hypnea cornuta* and *H. cervicornis* that "Stellate" propagules produced on the thallus exhibited regenerative properties. The profuse occurrence and the regeneration of the "Stellate" bulbils in *H. valentiae* and the "tendrils" in *H. musciformis* are taken advantage of employing them as "seed" material to culture these species by artificial means. In order to determine the regeneration capacity of the vegetative propagules in *Hypnea* sp. culture experiments were conducted both in the laboratory and field.

MATERIALS AND METHODS

Pieces of 1 cm in length were excised from the basal, middle and tendrillar regions of the healthy fronds of *H. musciformis* and cultured separately in Erdschreiber enriched seawater in northern diffused day light (Foyt 1934). 10 pieces in five replicates were used in each series. The growth of these pieces was measured in terms of fresh weight after two months. The culture medium was replenished every days.

In *H. valentiae* the seasonal periodicity in the production of "Stellate" bulbils was studied. From the plants collected at Pamban (Mandapam) at one week intervals for about eight months during January 1970 - April 1971 and the ratio between the plants with and without "Stellate" bulbils was determined. Some of the bulbils were allowed to shed on the shells of *placenta*. *Placenta* kept in a trough of filtered seawater

and grown in the laboratory for one and half months after ensuring firm attachment of these "Stellate" bulbils to the shells were transferred into the sea at Krusadai Island. The shells were tied to a nylon rope and this rope was suspended in the sea by tying to two bamboo poles fixed in the lagoon. The experiment was conducted for two months after which the shells were broken due to rough sea condition. The growth rate of the "Stellate" bulbils was measured in terms of increase in length at intervals of fifteen days. For this purpose, 10 shells were numbered with quick drying paint and the length of 5 "Stellate" bulbils on each one of these shells was recorded through out the experiment.

RESULTS

In *H. musciformis* no regeneration was found in the pieces excised from the basal region while the fragments from the middle part of the thallus and tendrills rapidly produced proliferations. These were more numerous and pronounced on the tendrills than on the middle fragments. There was approximately ten fold increase in the fresh weight in the tendrills as compared to very little increase in the other two fragments (Table 1).

Table 1: Growth of excised pieces from basal, middle and Tendrillar* regions of *Hypnea musciformis* in culture for two months.

Description of the excised portion	Initial fresh wt. (mean of 10 pieces) (mg)	Final fresh wt. (mean of 10 pieces) (mg)	Total increase in fresh wt. (mg)	Percentage increase in fresh wt. over initial
Basal	9.80	10.20	0.40	4.09
Middle	12.50	13.46	0.96	7.68
Tendrill	13.26	14.60	1.34	10.10

* Tendrillar Region: Healthy, stout and well grown tendrills were excised for the purpose of comparative growth between the three regions.

In *H. valentiae* the vegetative propagation taken place by "Stellate" bulbils. These were produced both on the cystocarpic and tetrasporic plants. The production of the "Stellate" bulbils showed seasonal variation from July 1970 up to April 1971 (Table 2). The percentage of plants having bulbils gradually increased from July 1970 until about 80% plants were bearing these bulbils in January 1971. There was a decrease in number of bulbils in February and March and a sharp fall in April 1971. These bulbils, when mature, drop off at the slightest disturbance and attached to the substratum, settled down and grew quickly into new plants. The settlement was facilitated by the three short arms. In general the growth of future thallus takes place from the longer arm of the bulbil. But in the laboratory conditions any or all of the arms of the bulbils developed into new plants. Sometimes stellate bulbils developed into large branches *in situ* on the parent plant. These branches anastomosed when they came into contact with one another. Sometimes, when the branches bearing the "Stellate" bulbils came in contact with any substratum, the bulbils developed into runners from which new axes arose by proliferation.

Table 2: Seasonal* periodicity in the incidence of "Stellate bulbils" in *Hypnea valentiae* at Pamban.

Month	No. of Plants observed	No. of Plants with stellate bulbils	No. of Plants without stellate bulbils	Percentage of Plants	
				with stellate bulbils	without stellate bulbils
July 1970	232	128	104	55	45
August	368	232	136	63	37
September	424	280	144	66	34
October	356	160	196	45	55
November	334	251	83	75	25
December	276	216	60	78	22
January 1971	389	317	72	81	19
February	349	271	78	77	23
March	380	288	92	70	30
April	420	160	266	38	62

*Average values of the data collected at intervals of one week were presented in the table.

The branching in the early stages of the bulbils on the shells was irregular, but later became typical of the species viz. alternate to subdichotomous. The growth of the "Stellate" bulbils in the laboratory was slow and a total increase of 266% in length was observed after 45 days of growth (Table 3). However quick growth was seen after the shells bearing the bulbils were transferred to the sea at Krusadai Island

and plants measuring 10 cm 20 cm were obtained within two months and a total increase of 219% in length was recorded (Table 4).

Table 3: Growth of "Stellate Bulbils" of *Hypnea valentiae* in the laboratory culture.

Shell number	Initial length of bulbil (mm)	Percentage increase in length of the bulbils after		
		15 days	30 days	45 days
I	1.5	53	100	180
II	1.8	50	61	100
III	1.7	71	188	151
IV	1.7	94	141	171
V	1.7	106	171	200
VI	1.5	107	180	268
VII	1.7	106	171	206
IX	1.4	36	107	224
X	1.5	33	93	167

Table 4: Growth of "Stellate Bulbils" of *Hypnea valentiae* in the sea at Krusadai Island.

Shell Number	Initial Length of bulbils in mm	Percentage increase in length of the bulbils after			
		15 days	30 days	45 days	60 days
I	4.2	428	912	1340	1923
II	3.6	490	910	1393	1994
III	4.4	608	727	1477	1906
IV	4.6	452	725	978	1447
V	5.1	218	525	1059	1170
VI	5.5	340	705	1144	1522
VII	5.2	221	569	055	1250
VIII	4.4	232	709	1018	1748
IX	4.5	440	898	1473	1893
X	4.3	409	1107	1700	2197

H. musciformis grows on a highly exposed coast of Veraval where surf action is severe. Therefore, tips of the branches assumed "Crozier" like structure and twined round the neighbouring alga *Laurencia pinnatifida*. Due to wave action these "Croziers" became severed from the rest of the plant, and sprouted to develop into new plants. Thus it acted as an organ of perennation and aided in the vegetative propagation of the plant.

Although regeneration of fragments of the thallus was reported earlier (Okamura 1911; Marshall *et al.*, 1949; Philisbury 1950; Stockey 1957; Austin 1960 and Oza 1971): Regeneration from a morphological entity like a "Crozier" branch is the first report.

In *H. valentiae* unlike in *H. musciformis*, vegetative propagation takes place by special vegetative reproductive structures

called "Stellate" bulbils. During the present investigation it was noticed that these "Stellate" bulbils were found to develop into new plants in the field at Krusadai Island and Pamban. Hence, the seasonal periodicity in their incidence and growth in the laboratory and in the field showed that production of these "Stellate" bulbils commenced from July and continued till next April.

The number of plants bearing "stellate" bulbils increased along with that of tetrasporophytes from August to January till both approximately reached their maximum values and the number of plants bearing these stellate bulbils was small during the cystocarpic season from April to August. Hence, it can be inferred that vegetative propagation of tetrasporophytes was more vigorous by means of the "stellate" bulbils than the cystocarpic plants and hence the frequency of tetrasporophytes in nature was greater than that of cystocarpic plants.

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