On the regeneration in Calobryales

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Kumar, D., Manocha, N. & Tiwari, P. 2000. On the regeneration in Calobryales. *Geophytology.* **29** (1&2) : 81-87.

The paper describes the occurrence and development of regenerants from the leaf cells of *Haplomitrium* and *Calobryum*, both *in vivo* and *in vitro*, for the first time in Indian bryology.

Key-words - Hepaticae; Calobryales; *Haplomitrium*; *Calobryum*, Regeneration.

INTRODUCTION

REGENERATION in nature can be regarded as an effective means of vegetative propogation (Olarinmoye, 1978) which enables the bryophytes to produce many new plants under optimal growth conditions. Generally, the more simple and undifferentiated a plant is, the higher is its regenerative capacity (Ahmad & Dagar, 1987). Though regenerants in liverworts are rare in nature, yet they regenerate with particular readiness and every cell is totipotent for the branch formation. For the purpose of regeneration, liverworts produce special gemmae or reproductive bodies or even certain vegetative areas which can secure propagation and which are activated by isolation from the apical part of the plants (Bertheir et al., 1976).

In some way or the other, various physical and chemical factors affect the reproductive biology of liverworts (Wann, 1925; Voth & Hammer, 1940; Burgeff, 1943; Benson-Evans, 1961, 1964; Chopra & Sood, 1973, 1973a; Udar, 1976). The rigours of nature and varied ecological conditions influence growth and fertilization. In dioecious plants, the chances of fertilization are relatively low due to separation of sexes and also because of the different periods of maturation in some plants. Under these restrictions, sexual reproduction is often not accomplished. Consequently, such taxa usually resort to modes of vegetative or asexual reproduction for their survival (Schuster, 1966.)

Calobryales, including two genera viz., *Haplomitrium* and *Calobryum* (but only one genus *Haplomitrium*: *sensu* Schuster, 1963) lack any device of vegetative propagation in nature. These are dioecious [except *Haplomitrium monoicum* which is monoecious and reported from New Caledonia (Engel, 1981)] and dimorphic with profusely branched rhizomatous axes giving rise to erect leafy shoots and downwardly directed root systems. When the spores of *Calobryum* prove ineffective to germinate in nature, its growth is maintained exclusively by rapid ramification of the subterranean rhizome, where the interwoven, compactly branched, leafless rhizomatous portion of the plant, periodically giving rise to erect leafy gametophores and downwardly directed geotropic leafless root systems (Grubb, 1970) and appears to dominate the sexual cycle. Even a detached portion of the colony proliferate to give rise to a profusely branched clone of the same type. This process thus appears to be the only method of multiplication in *Calobryum*, whereas *Haplomitrium hookeri* occurring both in eastern and western Himalaya (Udar & Singh, 1977; Udar, 1980) never (or occasionally) forms compactly branched axes like those in *Calobryum*. It, therefore, reproduces by means of spores, although there is no supporting evidence of spore germination in nature. Even in artificial conditions, the spores of *Haplomitrium* rarely germinate or if they germinate, the protonema usually dies early in development. Gottsche (1843) failed to germinate the spores of *Haplomitrium hookeri* on moistened sand. But in further attempts, the spores of other species of *Haplomitrium* showed successful germination, particularly, of *H. gibbsiae* on andesitic clay (Campbell, 1959), of *H. mnioides* on half strength Knop's solution (Nehira, 1961, 1966) but in both the cases growth ceased at the cell mass stage. Only Yang (1967) and Yang *et al.* (1968) were able to grow the sporelings of *H. mnioides* and *H. blumii*. Furuki (1986) re-

ported the occurrence of gemmae in *H. hookeri* collected from Yatsugatake mountains of central Japan. This is the first and the only report of gemmae among the Calobryales. Recently, Bartholomew-Began (1991) also reported the spore germination patterns for at least five species of *Haplomitrium* including *H. hookeri*. She also observed the regenerants on the leaves of *H. intermedium* and *H. mnioides* from vermiculite cultures and of *H. hookeri* from Hatcher medium, developing from cells of both the leaf margin and lamina. Therefore, under artificial conditions multiplication is achieved not only by means of the rhizomatous system acting as a perennating structure, but also through regeneration from leaf-cells.

On the contrary, in *Calobryum* regeneration has neither been observed in natural conditions nor been studied in culture conditions. In a recent collection of liverworts from Darjeeling, eastern Himalayas, both the taxa of Calobrayles viz., *Calobryum* and *Haplomitrium* have been found developing regenerants from the margin as well as from the surface of the leaf in natural conditions. These regenerants appearing like those of parental shoots, are supposed to be the means of vegetative or asexual propagation of Calobryalean elements in nature. The present paper thus includes the study of these regenerants in different developmental stages. Besides this, leaves were also subjected to experimental conditions of Knop's medium of varying strength and some of the early stages of cell differentiation have been observed. The occurrence and development of such type of regenerants on the leaves of Calobryalean taxa i.e. *Calobryum indicum* and *Haplomitrium hookeri* in natural conditions has not been reported earlier.

MATERIAL AND METHOD

Haplomitrium and *Calobryum* collected on way to Senchal lake range (*ca* 2240 m) from Ghoom, Darjeeling, eastern Himalayas, during the month of November, separated out from rest of the hepatics and observed critically under a binocular microscope. Species of both the taxa were identified as *Haplomitrium hookeri* and *Calobryum indicum*. *Calobryum indicum* grows characteristically in large compact patches in extremely shaded and moist situations in the interior or large boulders. Male and female plants forming separate patches. *Haplomitrium hookeri* also grows in moist and shady conditions but they form loose patches. The associates were different species of *Jungermannia*, *Scapania* and *Pogonatum*.

The plants and some of the detached leaves showing development of regenerants were also separated out and washed to remove all the soil particles and other contaminations. These were then subjected to Knop's solution of varying strength. An experimental study has also been carried out during the month of January and February when minimum and maximum average temperature were 7.3°C and 23.8°C respectively. Several leaves of *Calobryum indicum* were removed, some of which were complete and some were cut in transverse and longitudinal planes. These were then placed in petridishes containing 25%, 50% and 100% Knop's solution and the cultures were kept in diffused light of ten hours duration through north window pans of the laboratory and observations were taken regularly.

OBSERVATION

Regenerants growing in natural conditions were found in different developmental stages. These regenerants may develop on both the leaf surfaces and there is no definite or particular point as they show their growth from the distal region (Text-figure 1: Figs 2,

PLATE-1 →

(Figs 1-6. Under experimental conditions of 100% Knop's solution)

Fig. 1. Leaf cell showing initiation of regeneration . Figs 2, 3. Leaves showing various developmental stages of regenerants. Figs 4-6. Leaves showing many new regions of regeneration alongwith different developmental stages

(Figs 7-19. Under natural conditions)

Figs. 7, 8, 10, 15. Showing regenerants developing marginally from the upper portion of the leaf. Figs 9, 14. Regenerants from the marginal region of the leaf. Figs 10, 12. Showing well-developed regenerants from the upper portion of the leaf. Fig. 11. Showing regenerant from the submarginal region of the leaf. Fig. 13. Showing regenerant from the middle region of the leaf. Fig. 16. Showing lamellae like structure on the leaf surface. Fig. 17. Regenerants with well-developed axes 'R' on the leaf. Fig. 18. Regenerant with thick mucilage covering. Fig. 19. A leaf of plant showing well-developed regenerant bearing 3-4 leaves (a miniature plant).

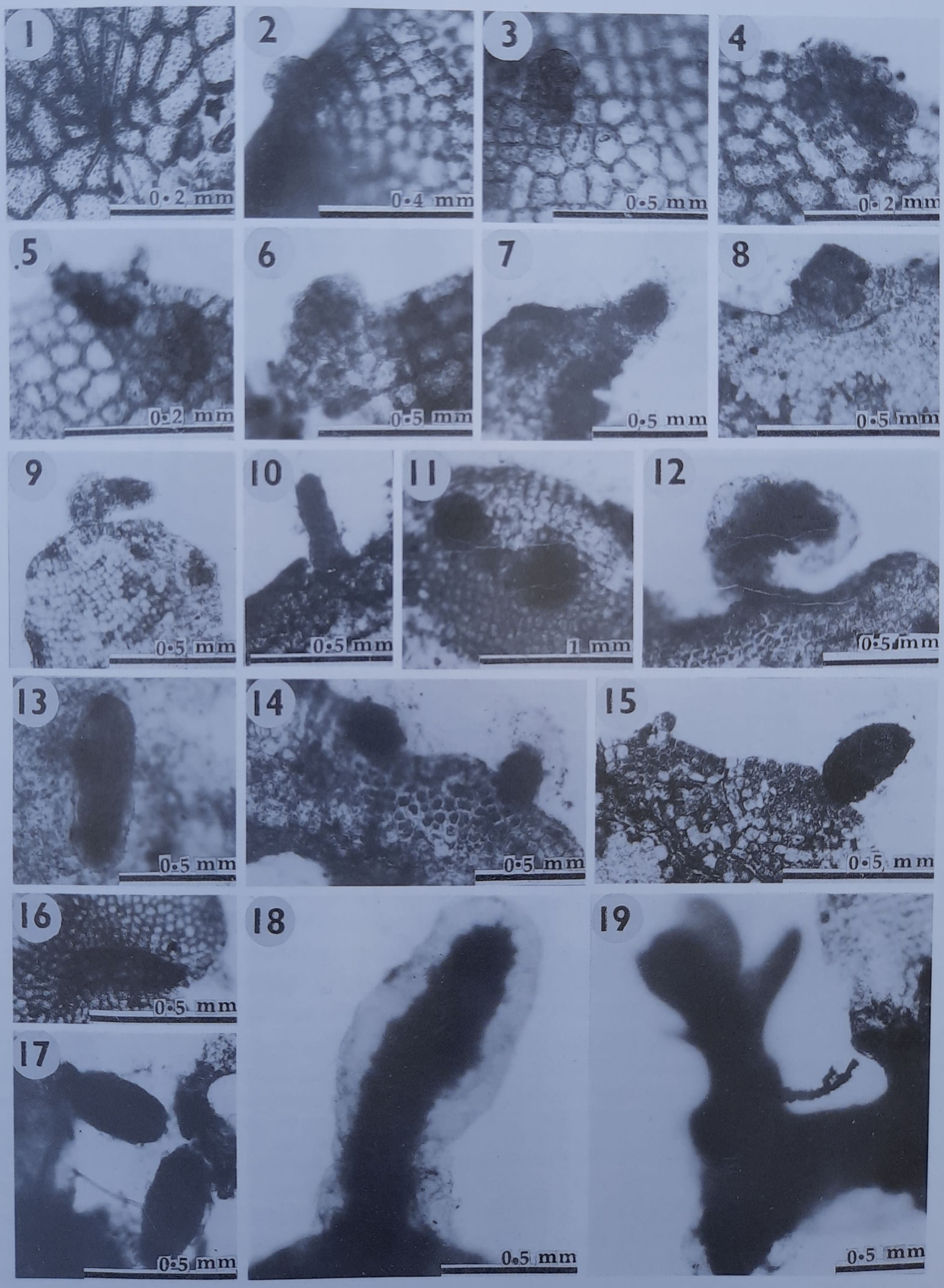
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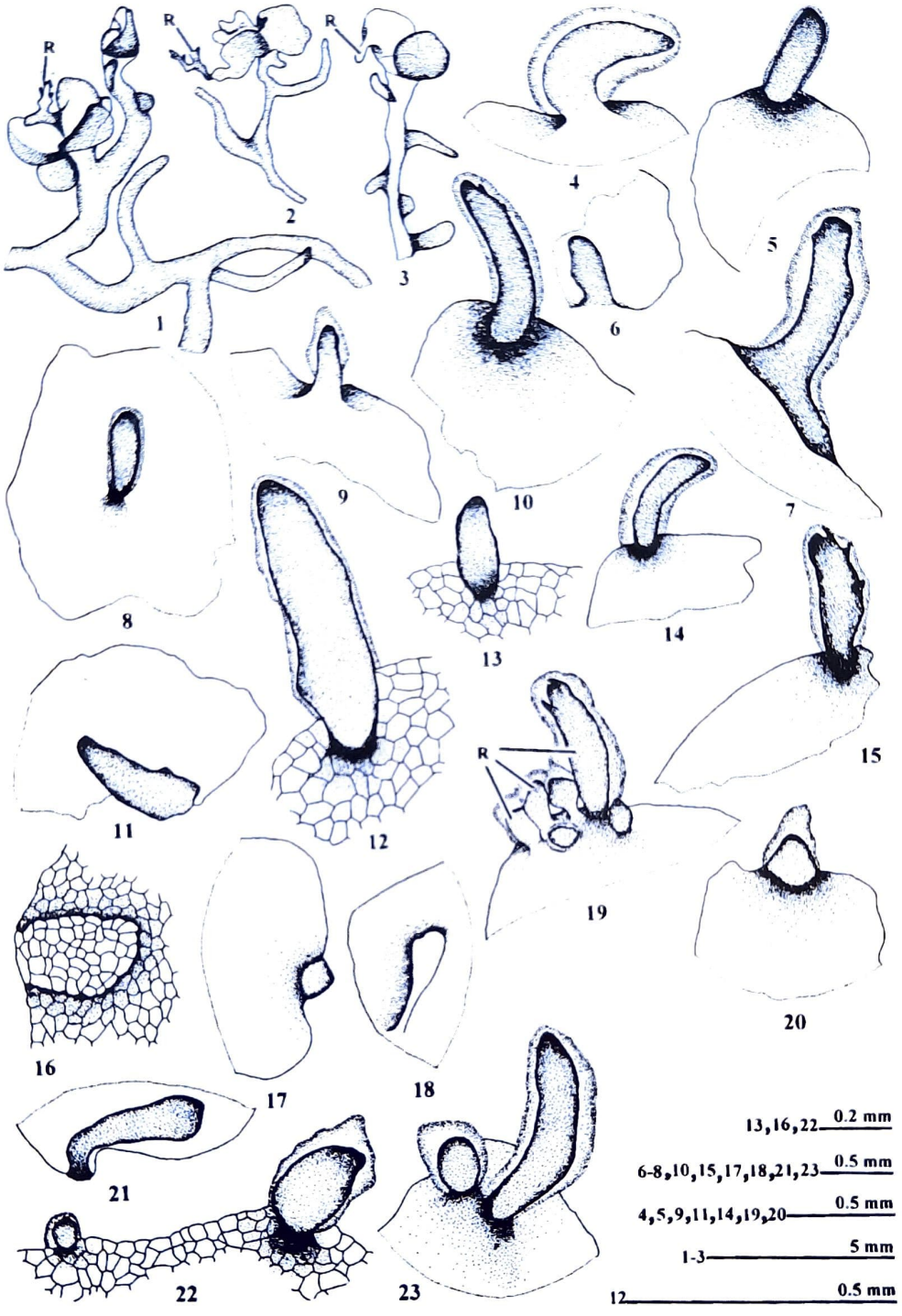
PLATE 1



Figs 1,2. Plants showing regenerants 'R' like a miniature plant on the leaf surface. Fig. 3. Young regenerant without leaves on the leaf surface. Figs 4, 9, 12, 13. Showing regenerants from the submarginal region of the detached leaves. Figs 5, 17, 20. Showing regenerants developing marginally on the upper half of the detached leaves. Figs 6, 11, 21. Showing regenerants developing from the basal region of the detached leaves. Figs 7, 10, 14, 15. Showing regenerants developing submarginally from the upper half of the detached leaves. Fig. 8. Showing regenerant from surface cells at the middle region of the detached leaf. Figs 16, 18. Leaf surface showing flap like tissue. Figs 19, 23. Showing number of regenerants with different developmental stages from the upper half of the detached leaf.

5, 7, 10, 14, 15, 19 and 20; Plate 1: Figs 7, 8, 10,12, 15 and 18), from the middle region (Text-figure 1: Fig. 8; Plate 1: Figs 3 and 13) as well as from the basal region of the leaf (Text-figure 1: Figs 6, 11 and 21; Plate 1: Fig. 19). Some regenerants grow exactly from the margins of the leaf (Text-figure 1: Figs 2, 5, 17, 20 and 22; Plate 1: Figs 6-9, 10,14, 15 and 17) whereas submarginal regenerants have also been observed (Text-figure 1: Figs 1, 3, 4, 7, 9, 10, 12-15, 19,22 and 23; Plate 1 : Figs 4, 11,18 and 19). Simi-

lar to the parent plant almost all the regenerants develop mucilaginous sheath around them (Text-figure 1: Figs 4, 5, 7-10, 12, 14, 15, 19,20,22 and 23; Plate 1: Figs 12-14, 18 and 19). Number of regenerants per leaf varies from 1-6 but their sizes vary on the same leaf (Text-figure 1: Figs 19,22 and 23; Plate 1: Figs 11,14, 15 and 17).

Size of the regenerants varies from 0.54-1.46 mm in length and 0.08-0.41 mm in thickness. Different patterns of growth and developmental stages result in variable shapes and forms of regenerants which may be like a protuberance (Text-figure 1: Figs 17 and 20; Plate 1: Figs 6-8), small, oval structures perpendicular to the leaf surface (Text-figure 1: Figs 19, 22, and 23; Plate 1: Figs 2-6,11,14 and 15), long thick, unbranched, straight or curved cylindrical, stout and erect structures (Text-figure 1: Figs 4-15, 19, 21 and 23; Plate 1: Figs 9,10,12,13, 17 and 18). Some of them also form 2-3 leaves towards apex (Text-figure 1 : Figs 1,2,10,15 and 19). Branched regenerants appearing like young plantlets developing on the leaves attached to the parent plants (Text-figure 1: Figs 1 and 2; Plate 1: Fig. 19) bearing 3-5 leaves thus show the regenerative capacity of young and green leaves besides brown and detached leaves.

Another kind of growth observed was in the form of unistratose layer of tissue forming a flap-like (Text-figure 1: Figs 16 and 18) or lamellae-like (Plate 1: Fig. 16) structure over the leaf surface. When leaves with naturally occurring regenerants were subjected to Knop's solution of varying strength, no further growth took place in the regenerants even after a period of six weeks. But when leaves of *Calobryum* cut in different planes and were given the same artificial conditions of nutrient medium and light, cell differentiation occurred only in the 100% Knop's solution after a period of four weeks (Plate 1: Fig. 1). Such differentiation was not restricted to a single cell in a leaf but many such type of regions developed on the same leaf (Plate 1: Fig. 4). The initiating cell divides (Plate 1: Fig. 2) and after passing through various states of division (Plate 1: Figs 3-5) results in the formation of a small regenerant (Text-figure 1 : Figs 6 and 7). The regenerating portions on the leaf surface are somewhat dark green in colour as compared to other leaf cells. No matter how the leaves are placed under artificial conditions, cells towards periphery or exactly at the margins develop the regenerative capacity in whole leaves and leaves cut transversely and no regeneration was observed at the cut ends of the leaves. Leaves which were cut longitudinally did not show any sign of regeneration.

DISCUSSION

The unisexual taxa of hepatics maintain their growth by gradual spreading from a single initial shoot presumbly derived from a single spore. Such a clone formation, occurring by branching of mature plant with subsequent death of older axes, often stimulated by decapitation or death of the apices of leading shoots, is an important factor in reducing the incidence of sexual reproduction. The Calobryalean taxa also spread by rapid ramification of rhizomatous axes but now the occurrence of regenerating shoots on the leaves of *Haplomitrium hookeri* and *Calobryum indicum* suggests another means of vegetative propagation thus showing a tendency towards regression of sexual reproduction as has already been observed in many other hepatics (Schuster, 1966; Longton, 1976).

In many cases species occur in entire large regions as one sex populations or sex-organs are rarely or never produced leading to wholly sterile population (Schuster, 1966, 1980). Such conditions enable the plants to adapt asexual reproductive devices for the survival and maintenance of the whole clone in the natural environment.

Among the various methods of vegetative propagation, leaf derived devices including gemmae (1-few celled), fragmenting or caducous leaves or leaf lobes/teeth and leaf cladia are very common in almost all the families of the order Jungermanniales except some of the primitive ones producing only gemmae. The species of these leafy hepaticae regenerate young plantlets from leaf cells of old and obviously moribund (or unhealthy) plants. However, it is difficult to draw a sharp distinction between such methods of reproduction through regeneration and those in which the leaves, even if healthy, regularly produce leafy propagula or leaf cladia (various species of *Plagiochila*) (Schuster, 1966).

The Metzgeriales, however, have evolved specialized asexual modes in several groups e.g. stalked pluricellular gemmae in *Xenothallus*, endogenous gemmae in *Riccardia*, discoid or thalloid gemmae in *Metzgeria* and have also developed tubers in *Fossombronia*, *Sewardiella* and *Petalophyllum* and two types of gammae in the members of Blasiaceae. Mehra (1976) found interesting results in his experiments on the regenerations of thalli of *Fossombronia himalayensis* and *Sewardiella tuberifera* where leaves (*Fossombronia*) or wings (*Sewardiella*) or their parts, isolated from the axes showed ample power of regeneration in 50% Knop's solution. Thus, leaves or wings can produce regenerants but only under artificial conditions.

In Calobryales, however, asexual reproduction by specialized bodies is absent (Schuster, 1966a) and only vegetative multiplication dominates. The first and the only report of the occurrence of specialized asexual bodies like gemmae are reported by Furuki (1986) in *Haplomitrium hookeri* collected from central Japan. These gemmae are multicellular globose structures developing on the uniseriate stalk arising from the cortical cells at the apex of the stem. However, these multicellular globose gemmae are present only in these plants of *Haplomitrium hookeri*. The plants of *H. hookeri* reported from the other localities do not show the occurrence of these gemmae. Even the recently collected plants of *H. hookeri* from Darjeeling did not show the occurrence of such type of gemmae. In addition to this, the other taxon of the order Calobryales do not show the occurrence of such type of gemmae. In the recent collection from Darjeeling, leaf cells of both the Calobryalean taxa i.e. *Haplomitrium* and *Calobryum* were found developing regenerants in the natural environment. The plants of *H. hookeri* and *C. indicum* have been collected so many times in the past from the same localities but such an unusual behaviour of leaf cells showing development of regenerating structures has not been observed.

The adaptation towards vegetative propagation is important where the taxa could not produce gametangia of one or both sexes but in the taxa where both sexes still survive with the sporophytes (like *Haplomitrium* and *Calobryum*) developed enough to produce spores, the purpose of such regeneration phenomenon is to bypass the sexual cycle and short-circuit the life-cycle. In this way, genetic recombinations involved in the sexual cycles are also omitted, as the only effective genetic change is in the somatic genes which may lead to the development of new biotypes.

So far as culture studies in Calobryales are concerned, the reports on the growth of *Haplomitrium* in organic medium include the experiments of Fulford & Diller, 1956 and Sharma *et al.*, 1960 (see also Ahmad & Dagar, 1987). Besides, spore germination in various species of *Haplomitrium* has also been studied by Campbell (1959), Nehira (1961, 1966), Yang (1967) and Yang *et al.* (1968). Recently, Bartholomew-Began (1991) described in detail the patterns of spore germination in five species of *Haplomitrium* including *H. hookeri*. She also observed the regenerants on the leaves of *H. intermedium* and *H. mnioides* from vermiculite cultures and of *H. hookeri* from Hatcher medium. But the other member of Calobryales i.e. *Calobryum* lacks any such kind of experimental records. In the present study, the positive response of leaf cells of *Calobryum* towards artificial conditions of nutrient medium, light and temperature suggests the adaptation towards adverse climatic conditions.

Thus, for the first time both the taxa of Calobryales i.e. *Haplomitrium* and *Calobryum* have been found to show the regenerating leaves with the development of few celled structures to young regenerants with few leaves, appearing like a young plant, arising from the margin as well as from the surface of the detached and attached young green to older brownish leaves of the parent plant in natural as well as in artificial conditions.

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(Received 31.05.2000; Accepted 29.09.2000)