Studies on the regeneration in *Riccia billardieri* Mont. *et* Nees and *Cyathodium aureonitens* (Griff.) Mitt.*

Dinesh Kumar, Pratima Srivastava** and Navita Manocha

Department of Botany, University of Lucknow, Lucknow-226 007

Kumar, D., Srivastava, Pratima & Manocha, Navita 1995. Studies on the regeneration in *Riccia billardieri* Mont. et Nees and *Cyathodium aureonitens* (Griff.) Mitt. *Geophytology* **24** (2):187-197.

The present paper deals with the regeneration studies on two taxa of liverworts i.e. *Riccia billardieri* Mont. *et* Nees and *Cyathodium aureonitens* (Griff.) Mitt. belonging to two different families Ricciaceae and Targioniaceae, respectively of the order Marchantiales. The thalli of both the taxa were subjected to four different conditions of nutrient medium of inorganic salts and both were found behaving differently under same experimental conditions.

Key-words- Bryophyta, Hepaticae, Marchantiales, Ricciaceae, Targioniaceae, *Riccia billardieri*, *Cyathodium aureonitens*.

INTRODUCTION

REGENERATION in bryophytes is an important phenomenon, not only providing protection to these non-vascular plants from the adversaries of nature but also results in the formation of a new individual by the activation of dormant buds or primordia. Regeneration in liverworts was first recognised by Necker (1774) by culturing various hepatics, like *Marchantia polymorpha*, *Conocephalum conicum*, *Riccia fluitans* and a number of leafy Jungermanniales, on soil. Later, Leitgeb (1879, 81) observed that ventral shoots are rare in *Marchantia* and *Conocephalum* but are common in *Plagiochasma* and *Riccia*. Vochting (1885) studied cell totipotency in the hepatics and concluded that polarity pervades all parts of the plant and each cell is totipotent in its capacity for regeneration and development. Under artificial conditions, formation of adventitious shoots from the thalli of *Riccia crystallina*, *Conocephalum conicum*, *Riccia fluitans*, *Ricciocarpus natans*, *Reboulia hemisphaerica* and *Preissia quadrata* has also been observed by Schostakowitsch (1894) and Bölleter (1905).

Udar (1957) also carried out experiments on regeneration in *Riccia crystallina* and stated that,"both the sporeling and regenerant broadly follow a similar pattern of developmental stages." He has also pointed out that in *Riccia*, earliest record in regeneration studies is that of *Riccia glauca* by Fellner (1875) who observed somewhat similar stages in regeneration as in *Riccia crystallina* and interpreted them to have originated by transformation of rhizoids but according to Udar (1957) that, "long tubes bearing the regenerants were mistaken for rhizoids." However, Mehra and Pahwa (1976) mentioned in their studies on regeneration of *Fossombronia himalayensis* Kash. that regenerants may be transformed rhizoids.

Sood (1972) also studied the effect of transverse and longitudinal cut on the pattern of regeneration in *Riccia crystallina* and found that portions with intact apices produced normal thalli by the activity of apical meristem whereas other cut parts showed regenerants all over the surface of the thalli and decapitated thallus could function as normal thallus with apical meristem. Further Chopra and Sood (1973) observed callus like tissue with abundant rhizoids in *Riccia crystallina* in the presence of 2 - 4 percent sucrose. Sood (1974) also observed the effects of mineral nutrients, chelates and organic nitrogenous substances on growth and sexuality in *Riccia crystallina*. Dagar (1974), Dagar and Ahmad (1976) and Ahmad and Dagar (1978) have investigated the effect of IAA, 2,4- D, NAA, GA3, 2,3,5-triiodobenzoic acid (TIBA) and cycocel (CCC) on regeneration of *Riccia discolor* and reported that any living vegetative cell is capable of regeneration. Vashistha and Chopra (1986) studied the effect of some physical and chemical factors on regeneration in the thalli of *Riccia frostii* and found that under different conditions of light, two types of regenerants were produced of

* Contribution New Series (Bryophyta) No. 249

** Present Address: Department of Botany, Faculty of Science, M.S. University of Baroda, Baroda.

which thalloid regenerants were formed mainly from injured portion of the plant, whereas filamentous regenerants were produced from the intact individual epidermal cells. Recently, Ahmad and Dagar (1987) has summarised the work on regeneration of Bryophyta providing the pattern and nature of regeneration and regenerants in all the major groups under artificial cultural conditions with adjuvants.

In the present work, two taxa of liverworts i.e. *Riccia billardieri* Mont. *et* Nees and *Cyathodium aureonitens* (Griff.) Mitt., belonging to the families Ricciaceae and Targioniaceae, respectively of the order Marchantiales, have been taken into consideration for the regeneration studies including percentage and behaviour of regenerants in nutrient medium of inorganic salts and for this purpose only Knop's solution was selected. The results of these investigations have been presented for the first time.

MATERIAL AND METHOD

Fresh and actively growing plants of *Riccia billardieri* Mont. *et* Nees and *Cyathodium aureonitens* (Griff.) Mitt. were collected from the garden of Botany Department, Lucknow University and Sindri Town of Dhanbad district in Bihar, respectively, during the month of October. Plants were thoroughly washed with tap water, then with sterilized distilled water to make them free from soil particles and other types of contamination. Corning glass petridishes of 4'' diameter were also washed thoroughly with water, then with sterilized water and finally they were flame sterilized. Several thalli of *Riccia billardieri* were taken as : a) whole thalli without any cut, b)cut longitudinally along the midrib, c) cut longitudinally with wings separated from midrib and d) cut transversely, whereas in *Cyathodium aureonitens:* a) cut was made transversely and b) whole thalli without any cut were taken. These thalli were placed on filter paper soaked in : a) water, b) soil extract, c) half strength Knop's solution and d) full strength Knop's solution in sterilized covered petridishes. The cultures were then placed in diffused light through north window panes of the laboratory and the day temperature ranged between 15 - 20°C. Observations were taken at an interval of 7 days for a period of 6 weeks. Filter papers in different petridishes were supplied the above nutrient media at regular intervals.

OBSERVATION

The thallus of *Riccia billardieri* is robust, compact, thick and dichotomously branched with prominent midrib. Internally, the thallus is differentiated into assimilatory zone consisting of assimilatory filaments and compact parenchymatous storage zone. Scales are present on the margin of the thallus and rhizoids of both types i.e. simple and tuberculate are situated on the midrib portion on the ventral side of the thallus. The thallus of *Cyathodium aureonitens* is also much dichotomously branched, thin and fan-shaped, without a distinct midrib i.e. having a large number of small veins on the whole thallus. Internally, the thallus is devoid of distinct storage zone and only assimilatory zone is present. Rhizoids are of simple and sinuous type. Ventral surface of the thallus is without ventral scales. Besides this there are other characters from which the thallus of *Riccia billardieri* differs from that of *Cyathodium aureonitens*.

Figures 1-59. ***Riccia billardieri*** **Mont. *et* Nees.** Figs 1-12: Regeneration in water. Figs 1-2: Whole thallus; fig 1. Regeneration from apical notch (3 weeks), fig 2. Regenerant (4weeks). Figs 3-6: Thallus cut longitudinally upto base along the midrib; figs 3,4. Initiation of bud at the upper cut ends (2 weeks), figs 5,6. Upturned tips and apical portion of the regenerants (3 weeks). Figs 7-8: Thallus cut longitudinally with wings separated from midrib; fig 7. Regeneration in the wings and in the apical notch region (2 weeks), fig 8. Dichotomy at the apex of the regenerant (3 weeks). Figs 9-12: Thallus cut transversely; fig 9. Initiating bud in the midrib region (2 weeks), fig 10. Initiation of bud in the upper cut part (3 weeks), fig 11. Appearance of dichotomy in the upper cut part (4 weeks), fig 12., Upturned tip of the regenerant (4 weeks). Figs 13-19: Regeneration in soil extract. Fig 13: Whole thallus; initiation of bud (3 weeks). Figs 14-19 : Thallus cut longitudinally upto base along midrib; fig 14,15. Initiation of regeneration behind the apical notch (2 weeks), figs 16,17. Initiation of buds on the cut parts (3 weeks), figs 18,19. Upturned tips and apical portion of the regenerants (4 weeks). Figs 20-22. Thallus cut longitudinally with wings separated from midrib; initiation of regeneration (3 weeks). Figs 23-25: Thallus cut transversely; fig 23. Initiation of bud from midrib region in lower half of the thallus (2 weeks), fig 24. Bud grow in size (3 weeks), fig 25. Regeneration in the apical notch region in upper half of the thallus (4 weeks). Figs 26-38: Regeneration in half Knop's solution. Figs 26-27: Whole thallus; fig 26. Initiation of bud (2 weeks), fig 27. Growth and development in bud (3 weeks). Figs 28-31: Thallus cut longitudinally upto base along the midrib; figs 28,29. Formation of bud at the cut part (2 weeks), figs 30,31. Development of an indistinct midrib in the buds (3 weeks). Figs 32-36: Thallus cut longitudinally with wings separated from midrib; fig 32. Regeneration in wings and apical notch region (2 weeks), figs 33,34. Development of an indistinct midrib and dichotomy (3 weeks), figs 35,36. Growth and development in regenerants (4 weeks). Figs 37-41: Thallus cut transversely; figs 37,38. Regeneration in both upper and lower halves(2 weeks), figs 39,40. Growth in the regenerants (3 weeks), fig 41. Appearance of dichotomy at the apex of the regenerant (4 weeks). Figs 42-59: Regeneration in full Knop's solution. Fig 42: Whole thallus; initiation of bud (3 weeks). Figs 43-46: Thallus cut longitudinally upto base along the midrib; figs 43,44. Regeneration behind or near the apical notch region (2weeks), figs 45,46. Regenerants with dichotomy at the apex (3 weeks). Figs 47-55: Thallus cut longitudinally with wings separated from midrib; figs 47-49. Initiation of regeneration from cut parts and midrib region (2 weeks), figs 50-52. Development of dichotomy and midrib in the regenerants (3 weeks), figs 53-55. Growth in regenerants (4 weeks). Figs 56-59: Thallus cut transversely; figs 56,57. Regeneration in both the cut halves (2 weeks), figs 58,59. Regenerants with distinct dichotomy (4 weeks).

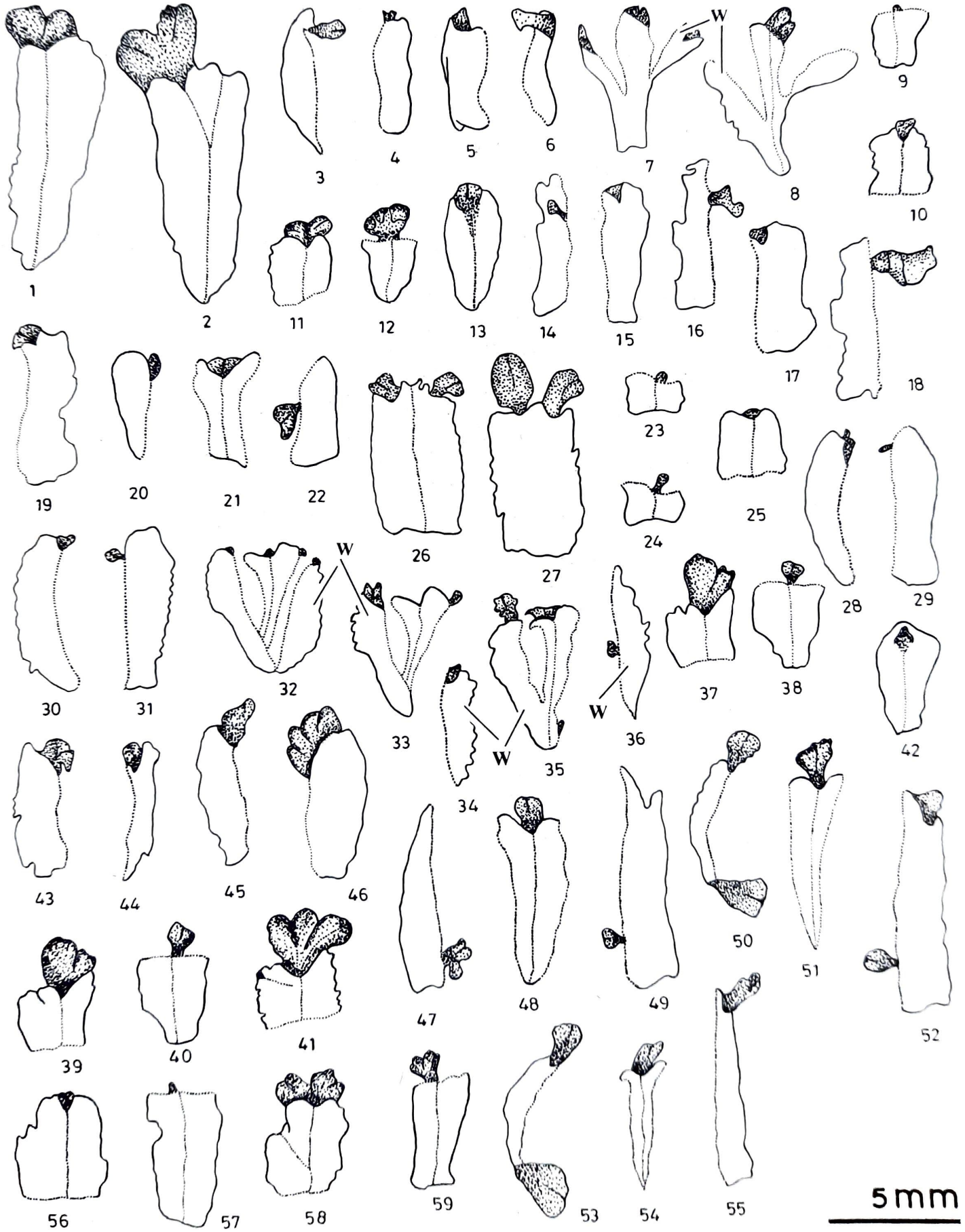

Fig. 1 - 59

Riccia billardieri Mont. *et* Nees

In *Riccia billardieri*, no meristematic activity could be observed in any condition of the thalli in any of the nutrient media after one week except that of half Knop's solution where it appeared only in the apical region of the transversely cut thalli. However, during this period, browning was observed in some cases, like in longitudinally cut thalli in half and full Knop's solution whereas whole thalli (in all nutrient media except soil extract) as well as transversely cut thalli (soil extract and full Knop's solution) showed browning after two weeks time. Whole thalli in soil extract and longitudinally cut thalli in water and soil extract remaind green throughout. The other observations regarding regeneration behaviour are as follows:

Whole Thallus: After two weeks time, the thalli kept in half Knop's solution showed meristematic activity resulting in the formation of a bud like structure from the apical notch (Fig. 26) (showing further growth and development in size after three weeks. Fig. 27) which develops minute scales (exhibiting violet colouration after four weeks time) and rhizoids of both simple and tuberculate type. After three weeks time, bud also initiated in the thalli kept in soil extract (Fig 13). The above features are noticeable in the regenerants of those thalli which were kept in other nutrient media, of which, the regenerants developed in full Knop's solution showed only simple rhizoids (Fig 42) whereas those regenerants developed in water showed rhizoids of both kinds (Fig 1). After four weeks time, the scales were observed in the regenerants developed in water (Fig 2) and there was no growth in the regenerants developed in soil extract and full Knop's solution (even after six weeks) except an increase in the percentage of regeneration with similar features (Table 1).

Thallus cut longitudinally along the midrib with wings separated at base: After the period of two weeks, meristematic activity initiated resulting in the formation of a bud like structure at the cut ends of the thalli in the upper part in water (Figs 3,4) and half Knop's solution (Figs 28, 29) whereas in soil extract and full Knop's solution meristematic activity initiated just behind or very near the apical notch (Figs 14, 15, 43, 44). This bud also showed the appearance of only simple rhizoids in water, both simple and tuberculate rhizoids in soil extract and even colourless scales in half Knop's solution. The same was observed in full Knop's solution after three weeks time, where the regenerants also exhibited dichotomy at their apex (Figs 45, 46) along with an increased growth and the development of an indistinct midrib in case of half Knop's solution (Figs 30, 31). During the same period, in water, regenerants developed tuberculate rhizoids and besides the growth of regenerants, initiation of some new buds on the thalli were also observed in soil extract (Figs 16, 17) and these buds developed purple coloured scales on their ventral side. In water, however, due to the increased growth of the regenerants, the tips and apical portion of the regenerants become upturned (Figs 5, 6), the phenomenon, which was noticed in soil extract after four weeks time (Figs 18, 19). This is the period during which percentage of regeneration was increased in every set of media (Table 1). Besides this, regenerants in water developed colourless scales whereas in full Knop's solution scales developed in three weeks time showed violet colouration.

Thallus cut longitudinally with wings separated from midrib: After the period of two weeks, the thalli kept in all nutrient media except soil extract showed meristematic activity from the upper cut part of the wings as well as from the apical notch region (Figs 7, 32, 47, 48, 49) along with regeneration in decayed parts in full Knop's solution. The regenerants during this period also showed initiation of both simple and tuberculate rhizoids in half and full Knop's solution but only simple rhizoids in water, whereas the tuberculate rhizoids became differentiated after three weeks time. During the same period, meristematic activity initiated in the thalli kept in soil extract (Figs 20, 21, 22) in the same manner and with similar features as in the thalli of other nutrient media. The regenerants developed in other nutrient

Figures 60-89. ***Cyathodium aureonitens*** **(Griff.) Mitt.** Figs 60-65: Regeneration in water. Figs 60-61: Whole thallus; fig 60. Bud initiation at the margin (1 week), fig 61. Development of new regenerants (4 weeks). Figs 62-65: Thallus cut transversely; figs 62,63. Mounds of tissue at the apex and cut ends in both the halves (2 weeks). figs 64,65. Growth in the regenerants (4 weeks). Figs 66-71: Regeneration in soil extract. Figs 66-67: Whole thallus; figs 66. Initiating buds from the margin (1 week), fig 67. Development of new regenerants (3 weeks). Figs 68-71: Thallus cut transversely; figs 68,69. Regeneration at the apex and cut end in both the cut halves (2 weeks), figs 70,71. Increased size of regenerants (3 weeks) Figs 72-80: Regeneration in half Knop's solution. Figs 72-75: Whole thallus; fig 72. Regeneration on the margin (1 week), fig 73. Regenerants from marginal and dorsal surface (2 weeks), fig 74. Regenerants with definite shape of the thallus (3 weeks), fig 75. Overlapped regenerants (4 weeks). Figs 76-80: Thallus cut transversely; fig 76. Regeneration towards margin in the upper half of the thallus (1 week), fig 77. Growth in the regenerants (2 weeks). fig 78. Regeneration at the cut surface in the lower half of the thallus (2 weeks), figs 79, 80. Growth in the regenerants in both the cut halves (4 weeks). Fig 81-89: Regeneration in full Knop's solution Figs 81-83: Whole thallus; fig 81. Initiating buds all around the thallus (1 week), fig 82. Increased size of regenerant (2 weeks), fig. 83. Regenerants with definite shape of the thallus (3 weeks). Figs 84-89: Thallus cut transversely; figs 84,85. Regeneration from dorsal and marginal surface in both the cut halves (3-4 days), figs 86,87. Growth in the regenerants and buds developed from the margin in both the halves (2 weeks), figs 88,89. Development of dichotomy in the regenerants in both the cut halves (4 weeks). (Shaded portions in the figures show regeneration or regenerants. 'w' stands for lateral wings.)

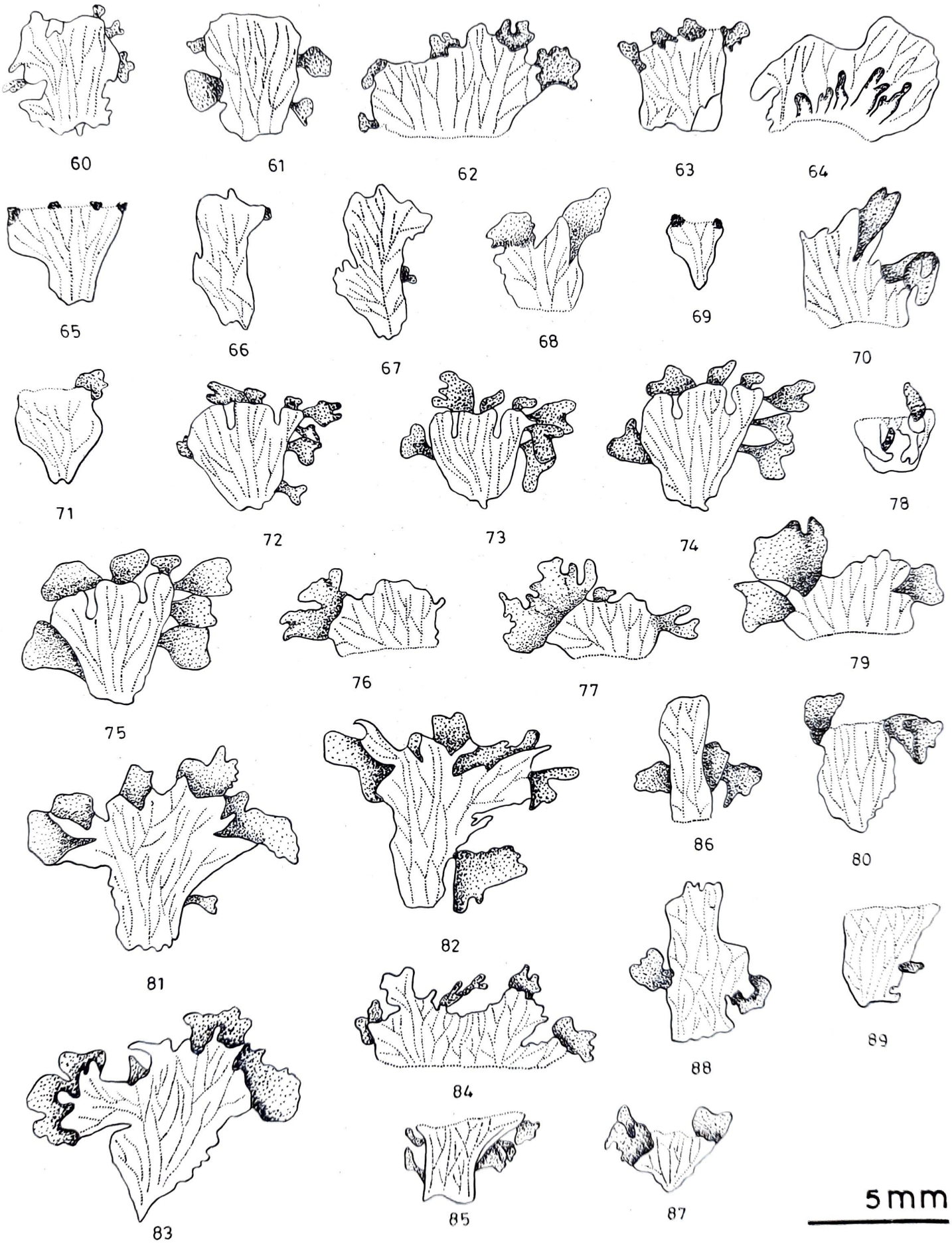
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
78
75
76
77
79
86
80
81
82
88
89
84
85
87
83
5mm

Fig. 60 - 89

Table 1: *Riccia billardieri* Mont. et Nees

NUTRIENTS			WATER					SOIL EXTRACT					HALF KNOP'S SOLUTION					FULL KNOP'S SOLUTION				
Thalli Characters		Weeks	Regeneration Percentage	Rhizoids		Scales		Regeneration Percentage	Rhizoids		Scales		Regeneration Percentage	Rhizoids		Scales		Regeneration Percentage	Rhizoids		Scales	
				Ini.	Type	Ini.	Type		Ini.	Type	Ini.	Type		Ini.	Type	Ini.	Type		Ini.	Type	Ini.	Type
Whole Thallus		I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-	-	+	+	S&T	+	H	-	-	-	-	-
		III	40	+	S&T	-	-	20	-	-	-	-	40	+	S&T	+	H	20	+	S	-	-
		IV	40	+	S&T	+	H	60	-	-	-	-	60	+	S&T	+	V	40	+	S	-	-
Thallus cut longitudinally along the midrib with wings separated at base		I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		II	20	+	S	-	-	20	+	S&T	-	-	60	+	S&T	+	H	40	-	-	-	-
		III	40	+	S&T	-	-	40	+	S&T	+	V	60	+	S&T	+	H	40	+	S&T	+	H
		IV	40	+	S&T	+	H	60	+	S&T	+	V	80	+	S&T	+	H	40	+	S&T	+	V
Thallus cut longitudinally from the midrib		I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		II	20	-	-	-	-	-	-	-	-	-	80	+	S&T	-	-	80	+	S&T	-	-
		III	20	+	S&T	-	-	60	+	S&T	-	-	80	+	S&T	-	-	80	+	S&T	+	H
		IV	20	+	S&T	-	-	60	+	S&T	-	-	100	+	S&T	+	H	100	+	S&T	+	V
Thallus cut trans-versely	Upper half	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		II	-	-	-	-	-	-	-	-	-	-	100	+	S&T	+	H	+	+	S&T	-	-
		III	+	-	-	-	-	-	-	-	-	-	100	+	S&T	+	H	40	+	S&T	+	H
		IV	+	-	-	-	-	+	-	-	-	-	100	+	S&T	+	V	60	+	S&T	+	V
	Lower half	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		II	40	+	S	-	-	20	-	-	-	-	100	+	S&T	+	H	+	+	S&T	-	-
		III	40	+	S	-	-	20	+	S&T	-	-	100	+	S&T	+	H	40	+	S&T	+	H
		IV	60	+	S	-	-	20	+	S&T	-	-	100	+	S&T	+	V	60	+	S&T	+	V

Abbreviations: Ini. : Initiation; S: Simple; T : Tuberculate; H: Hyaline; V: Violet; – : Absent; +: Present.

media like water, half and full Knop's solution also showed further growth with the development of distinct midrib and dichotomy at their apex (Figs 8, 33, 34, 50, 51, 52). After four weeks no remarkable change was observed. However, there was an increase in the percentage of regeneration (Table 1) and also the development of several new buds i.e. increase in the number of buds on the single thallus along with the further growth in earlier developed regenerants in the thalli kept in half and full Knop's solution (Figs 35, 36, 53, 54, 55). Besides this, colourless and violet coloured scales were also observed in the regenerants developed in half and full Knop's solutions respectively.

Thallus cut transversely : After two weeks time, in water and soil extract, only lower cut parts of the thalli showed meristematic activity in the form of a bud like structure in the midrib region (Figs 9, 23) whereas in half and full Knop's solution, both the cut halves showed regeneration in this period (Figs 37, 38, 56, 57). The regenerants developed in water showed initiation of few simple rhizoids also, whereas both simple and tuberculate rhizoids in full Knop's solution and even colourless scales initiated in the regenerants of half Knop's solution. The same was observed in the regenerants of full Knop's and soil extract solution after three weeks time, the period during which the upper cut part of the thalli also showed meristematic activity in water (Fig 10) (observed in soil extract after four weeks, Fig 25). No other remarkable change was observed in other nutrient media except an increase in the percentage of regeneration (Table 1) in full Knop's solution and increase in the length of rhizoids and regenerants in soil extract (Fig, 24) and half Knop's solution (Figs 39, 40) in three weeks time. Dichotomy appeared at the apices of regenerants in upper cut part only in water (Fig, 11) and half Knop's solution (Fig, 41) and in both the cut halves in full Knop's solution (Figs, 58, 59) after four weeks time and earlier developed colourless scales in the regenerants of half and full Knop's solution showed violet colouration. Other changes observed are increase in the number and size of the regenerants and rhizoids in water, half and full Knop's solution but in water, regenerants increased to the extent that they get upturned on themselves (Fig 12).

Cyathodium aureonitens (Griff.) Mitt.

Whole Thallus: In full Knop's solution meristematic activity was observed after four days in the form of dark green areas which ultimately developed into a small bud like structure (Fig 81) whereas in other nutrient media, it initiated after one week. During this period, the thalli kept in water showed the development of regenerants on the margins (Fig 60) while in soil extract, the lower part of the thalli showed browning and upper part after becoming dark green developed buds from the margin which exhibit indistinct dichotomy at its apex (Fig 66). The thalli kept in half Knop's solution showed the appearance of simple and sinuous rhizoids on the regenerants (Fig 72). The regenerants with same features have been observed in two weeks duration in those thalli which were kept in full Knop's solution (Fig 8). During this two weeks time, the regenerants developed only few simple rhizoids in soil extract and water. Similarly, the thalli, kept in half Knop's solution showed much vigorous development of regenerants appearing from the dorsal surface in addition to marginal regenerants (Fig 73). After the period of three weeks time regenerants further grew and developed distinct dichotomy at their apices thus attaining definite shape of the small thallus particularly in half (Fig 74) and full Knop's solution (Fig 83). Some new regenerants also developed during this period in soil extract (Fig 67) which are observed after four weeks time in water only (Fig 61). No further change was observed in full Knop's solution whereas in half Knop's solution regenerants increased in dimension to the extent that they start overlapping each other (Fig 75) and each regenerant developed rhizoids on its ventral side. After six weeks, the thalli, kept in half Knop's solution also developed sporophytes at the notches which were never observed in the regenerants developed in different sets of media.

Thallus cut transversely : In full Knop's solution, meristematic activity initiated afer 3 - 4 days in both the cut halves of the thalli. The upper cut part developed regenerants from the dorsal and marginal surface (Fig 84) whereas in lower part the bud originated from the margins of the thallus (Fig 85). The thalli kept in half Knop's solution showed meristematic activity in the form of dark green patches towards the margins of the thalli only in the upper cut part (Fig 76) whereas in water and soil extract, no visual activity was observed in the thalli kept in water during this period except browning which was seen after two weeks time in soil extract alongwith meristematic activity in the form of dark green portions towards apices of the upper cut part of the thalli (Fig 68) and mounds of tissue from the margin near the lower cut end of the thalli (Fig 69). In water, after two weeks duration, upper half of the thalli showed mounds of tissue at the apex (Fig 62) and regenerants from the lower cut half of the thalli in which some of the regenerants developed rhizoids (Fig 63) whereas in half and full Knop's solution rhizoids of both simple and sinuous types initiated during this period along with more growth of the regenerants in size as well as in number i.e. percentage of regeneration increased (Table 2) (Figs 77, 78, 86, 87). The same was observed in the thalli kept in soil extract after three weeks time (Figs 70, 71), the period during which no

Table 2: *Cyathodium aureonitens* (Griff.) Mitt.

NUTRIENTS			WATER			SOIL EXTRACT			HALF KNOP'S SOLUTION			FULL KNOP'S SOLUTION		
Thalli Characters		WEEKS	Regeneration Percentage	Rhizoids		Regeneration Percentage	Rhizoids		Regeneration Percentage	Rhizoids		Regeneration Percentage	Rhizoids	
				Ini.	Type		Ini.	Type		Ini.	Type		Ini.	Type
Whole Thallus		I	20	-	-	20	-	-	80	+	S&S	60	-	-
		II	20+	+	S	40	+	S	80	+	S&S	80	+	S&S
		III	40	+	S	60	+	S	80	+	S&S	80	+	S&S
		IV	60	+	S	60	+	S	80	+	S&S	80	+	S&S
Thallus cut transversely	Upper half	I	-	-	-	-	-	-	100	-	-	40	-	-
		II	60	-	-	+	-	-	100	+	S&S	60	-	-
		III	60	-	-	60	+	S&S	100	+	S&S	80	-	-
		IV	80	-	-	60	+	S&S	100	+	S&S	80	-	-
	Lower half	I	-	-	-	-	-	-	100	-	-	40	-	-
		II	60	+	S	+	-	-	100	+	S&S	60	+	S&S
		III	60	+	S	60	+	S&S	100	+	S&S	80	+	S&S
		IV	80	+	S	60	+	S&S	100	+	S&S	80	+	S&S

Abbreviations : Ini. : Initiation; S: Simple; S&S : Simple and sinuous; – : Absent; + : Present.

other remarkable change was observed in other media except further growth in earlier developed regenerants. After the period of four weeks, regenerants showed growth in half (Figs 79, 80) and full Knop's solution (Figs 88, 89) and an increase in the percentage of regeneration in the thalli kept in water (Table 2) (Figs 64, 65) but there was no change in the thalli kept in soil extract.

DISCUSSION

So far as regeneration in *Riccia billardieri* Mont. *et* Nees and *Cyathodium aureonitens* (Griff.) Mitt. in four different conditions, viz., full Knop's solution, half Knop's solution, water and soil extract, is concerned, maximum response with regard to initiation, development, growth of bud or regenerants is found in half Knop's solution. This is followed in sequence by water, Knop's solution and then soil extract in *Riccia billardieri* whereas in *Cyathodium aureonitens*, the sequence is half Knop's solution, full Knop's solution, water and then soil extract. Percentage of regeneration and rhizoids formation in the two taxa were also observed maximum in half Knop's solution. Even when the thalli were cut transversely, regeneration and growth were maximum in half Knop's solution in both the taxa. Initially, full Knop's solution proved best for the initiation of regeneration but later on half Knop's solution was found to be the best for the development and growth of regenerants. In *Riccia billardieri*, the half Knop's solution is suffficient to induce regeneration in healthy apical part and cut parts of the thallus but it shows its inability in producing regenerants from decaying parts of the thallus. However, the decaying wings of longitudinally cut thalli kept in full Knop's solution were able to produce regenerants. The reason for this may be more mineral requirement in the decaying tissue than the normal tissue of the thallus, which is fulfilled by full Knop's solution. This was not observed in soil extract and water.

In *Riccia billardieri*, development is very slow whereas in *Cyathodium aureonitens*, regenerants take lesser time to develop as it is clear from the observations. Besides, *Cyathodium aureonitens*, being monoecious, showed development of sex-organs, i.e. antheridia and archegonia on the regenerants, a kind of miniature of the thallus and since these have been supplied nutrient solution regularly, the regenerants also show the production of sporophytes at an interval of six weeks in half Knop's solution only, which are smaller in size and therefore, the size of spores and elaters remain smaller than those observed in normal thallus. The sex-organs and sporophytes formation was never observed in the regenerants of *Riccia billardieri* in any of the nutrient media.

So, it can be said on the basis of observations that in half Knop's solution, the percentage of regeneration as well as the number of regenerants were maximum. Mehra and Pahwa (1976) have also found that half Knop's solution proved best for the germination of sporelings of *Fossombronia himalayensis* Kash.

In their experiment, full Knop's solution proved next in sequence for germination of sporelings. It is thus deduced that nutrients required for germination of sporelings were similar to that required for origin and growth of regenerants from artificially isolated parts in *Riccia billardieri* and *Cyathodium aureonitens*, two liverworts under present investigation. In water, although regenerants were produced and growth also occurred in them due to presence of endogenous synthesis of mineral nutrients and growth regulators in the thallus. In soil extract, origin and growth of regenerants was slower than full Knop's and half Knop's solutions but greater than water for both the taxa, thus showing similarity in this feature. Sood (1974) also reported similar observations for *Riccia crystallina*, in which she used soil extract solidified with agar as a medium for growth of thalli's part and observed that, "explant showed very little growth and sometimes developed one or two regenerants."

In *Cyathodium aureonitens*, the thallus which is morphologically less differentiated internally than *Riccia billardieri*, there is no distinct polarity in regeneration, a considerable number of regenerants being formed at random from any place on the margin in half Knop's solution, water and soil extract or on the surface in full Knop's solution. So in *Cyathodium aureonitens*, regenerants are produced from both dorsal and ventral margin of the thallus. These results are in agreement with those of Allsopp and Ilahi (1970) while working with regeneration patterns on *Noteroclada confluens* and also from those of Kreh (1909) on *Clevea hyalina* who concluded that shoot could arise anywhere on the thallus although there was some preference for the ventral margin. In *Riccia billardieri* regenerants prefer to develop on the ventral margin. However, Pearman (1964) worked on regeneration in Marchantiales using some species of *Riccia* including *Riccia gangetica*, *Riccia sorocarpa*, *Riccia waronstorfii*, *Riccia fluitans*, *Riccia beyrichiana*, *Riccia crozalsii* along with other liverworts like *Marchantia polymorpha*, *Preissia quadrata*, *Dumortiera hirsuta*, *Monoselenium tenerum*, *Conocephalum conicum*, *Lunularia cruciata*, *Reboulia hemisphaerica*, *Plagiochasma rupestre*, *Corsinia coriandrina*, *Oxymitra paleacea* and specified 'Riccia type' regeneration where new shoots were formed from the cut surface either ventrally or dorsally in the centre of the midrib. In this type, approximately 1/3rd of the experimental thalli produced shoots from the dorsal surface only, another 1/3rd produced shoots

from the ventral surface only and the remaining 1/3rd produced shoots from both dorsal and ventral surface. Occassionally, shoots could be seen arising from dorsal surface of the plant in the midrib region some distance from the cut surface. This 'Riccia type' response was characteristic of *Oxymitra* and of almost all the species of *Riccia* with the exception of *Riccia fluitans*. In *Riccia billardieri*, regenerants were formed preferentially on the longitudinally or transversely cut ends of the thallus thus approaching the results of Pearman (1964) obtained for different species of *Riccia* and other hepatics. However, the regenerants were not always restricted to the cut surface in *Cyathodium*. So far as longitudinal cuts in *Riccia* are concerned, when the thallus was cut along the midrib throughout the length, regenerants were produced from the cut portions as according to the hypothesis made by Schostakowitsch (1894) that the "site of regeneration is determined by the direction of normal nutrient flow in the unwounded plant ", nutrients become accumulated at the cut part resulting in the production of regenerants. When the thalli were cut with their wings separated from midrib region, the apical part of the midrib, having apical meristem, grow in that direction without any production of regenerants from its cut ends. This was due to the inhibitory influence of apical cell, which does not allow growth of regenerants from cut end, whereas the cut ends of the wings, developed regenerants from both upper and lower portions, as they were lacking apical cell as well as nutrient flow, which occurred through midrib. These observations were similar to that of Vochting (1885) on his studies of the hepatic *Lunularia*, where he noticed that when cuts were made, so that only lateral lobes were removed, the apex being left intact, there was no replacement of lobes, but apex continued growth as a narrow beak which gradually broadened.

When whole thallus was investigated, no regenerants were produced from the lateral sides in *Riccia billardieri* due to inhibitory influence of apical cell whereas in *Cyathodium aureonitens*, there is no particular position of apical cell and thallus develops meristematic activity at the upper portion along with some regenerants from the lateral sides.

Therefore, it can be concluded that polarity is more, in evolved Marchantiales than those having less differentiated organization. The thallus of *Cyathodium aureonitens* show more similarity with Metzgeriales (Mehra, 1976). Greater polarity shown by Marchantiales is possibly connected with the nature of uptake of water and mineral nutrients. Most Marchantiales are endohydric forms which are characterised by an active transpiration stream, with uptake of water and nutrients by the well developed rhizoidal system and with well defined prominent midrib (Buch, 1947). The phenomenon of apical dominance occurred here due to the polarised flow of endogenous auxin from the apex, and in *Riccia billardieri* not only the removal of apex but longitudinal cuts of the thalli also resulted in the production of regenerants.

In higher plant tissues, callus formation usually requires the presence of fairly high concentration of hormones including not only auxin but frequently also cytokinins. Under present investigation, best results of regeneration for both the taxa were observed in half strength Knop's solution but the differences between these two taxa are that *Cyathodium aureonitens* showed an early response to Knop's solutions as compared to *Riccia billardieri* as well as it is also capable of developing sporophytes after six weeks. So far as regeneration pattern is concerned, another difference between the two taxa is that *Riccia billardieri* showed polarity in the lower cut end of the thallus whereas *Cyathodium aureonitens* showed lateral development of regenerants from the lower cut end of the thallus.

Development of regenerants in the thalli from both dorsal and ventral surface as well as from the lateral margins is a common feature of *Cyathodium aureonitens* due to the indefinite position of apical cell thus not limiting the regeneration as in case of *Riccia billardieri* where there is an inhibitory action of apical cell on the regeneration pattern. Though both the taxa behave differently yet an inorganic nutrient medium in the form of Knop's solution (particularly 50%) was found sufficient to induce regeneration in these two taxa without any growth regulator, inhibitor, carbohydrates and vitamins.

AKNOWLEDGEMENT

The authors are indebted to Dr. S.C.Srivastava, Botany Department, Lucknow University for going through the manuscript and valuable suggestions.

REFERENCES

Ahmad, S.M. & Dagar, J.C. 1978. Effect of auxins and antiauxins on regeneration of *Riccia discolor* L. & L. *J. Indian bot. Soc.* **57**: 228-232.

Ahmad, S.M. & Dagar, J.C. 1987. Regeneration in Bryophyta. *Indian Rev. Life Sci.* **7**: 89-115.

Allsopp, A. & Ilahi, I. 1970. Studies in the Metzgeriales (Hepaticae). III. The effect of sugars and auxins on *Noteroclada confluens*. *Phytomorphology*. **20**: 9-16.

Bölleter, E. 1905. *Fegatella conica* (L.) Corda. Eine morphologischephysiologische Monographie. *Beih. Bot. Centralbl.* **18**: 327-408.

Buch, H. 1947. De l' adaptation des bryophytes, des algues et des lichens aux conditions d'humidité et de la concentration de la solution nutritive dans les milieux naturels. *Revue. bryol. Lichen.* **16**: 54-59.

Chopra, R.N. & Sood, S. 1973. *In vitro* studies in *Marchantiales*. I. Effect of some carbohydrates, agar, pH, light and growth regulators on

the growth and sexuality in *Ricia crystallina*. *Phytomorphology*. **23**: 230-244.

Dagar, J.C. 1974. *Effect of some growth regulating substances on regeneration of Riccia discolor* L. & L. M.Sc. Thesis, Univ. of Bhopal, Bhopal.

Dagar, J.C. & Ahmad, S.M. 1976. Morphogenetic effects of an auxin on regeneration of *Riccia discolor*. *Botanique*.7:223- 225.

Fellner, F. 1875. Keimung der sporen von *Riccia glauca*. *Jber. akad. naturw. Ver., Graz.*, I: Jahrgang: **1:** 41-46.

Kreh, W. 1909. Über die Regeneration der Lebermoose. *Nova. Acta. Acad. Caesar. Leop.-Carol.* **90**: 213-301.

Leitgeb, H. 1879. Untersuchungen über die Lebermoose. IV. Die Ricciea: 1-101. *Graz.*

Leitgeb, H. 1881. Untersuchungen über die Lebermoose. VI. Die Marchantieen: 1-158.*Graz.*

Mehra, P.N. 1976. Regeneration in *Fossombronia himalayensis* Kash. and *Sewardiella tuberifera* Kash. *J. Hattori bot. Lab.* **40**: 521-532.

Mehra, P.N. & Pahwa, M.S. 1976. Phenotypic variations in *Fossombronia himalayensis* Kash.-*in vitro* effect of sugars, auxins, growth substances and growth inhibitors. *J. Hattori Bot. Lab.* **40** : 371-395.

Necker 1774. "*Physiologia Muscorum*". Page 27.

Pearman, C. 1964. *Morphogenetic studies in Hepaticae*. M.Sc. Thesis, Univ. of Manchester, Manchester.

Schostakowitsch 1894. Über die Reproductions- und Regenerations-Erscheinungen bei den Lebermoosen. *Flora . Jena.* **79**: 350-384.

Sood, S. 1972. *Experimental studies on some Indian bryophytes*.Ph.D. Thesis,University of Delhi, Delhi, India.

Sood, S. 1974. *In vitro* studies in *Marchantiales*-II. Effect of mineral nutrients, chelates and organic nitrogenous sources on the growth and sexuality in *Riccia crystallina*. *Phytomorphology*. **24**: 186-197.

Udar, R. 1957. Culture studies in the genus *Riccia* (Mich.) L. I.sporeling germination in *Riccia billardieri*. Mont. et N. *J. Indian bot. Soc.* **36** : 46-50.

Vashistha, B.D. & Chopra, R.N. 1986. Experimental studies on bryophytes in India -II. *Phytomorphology*. **36**(3,4): 299- 313.

Vochting, H. 1885. Ueber die Regeneration der Marchantieen. *Jb. Wiss. Bot.* **16** : 367-414.