MORPHOLOGICAL AND EMBRYOLOGICAL STUDIES OF SOME PODOSTEMACEAE MEMBERS OF NORTH EASTERN INDIA

ABSTRACT

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THESIS SUBMITTED IN PARTIAL FULFILMENT OF
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ABSTRACT

Podostemaceae Rich. Ex C. Agardh is the largest family of strictly aquatic flowering plants (Philbrick & Novello, 1995; Cook, 1996) and consists of aquatic angiosperms that typically grow on rocks in cascades, waterfalls and rapids where there are great fluctuations in the river water levels. They grow firmly attached to rocks and stones by means of adhesive holdfast or haptera, which secretes mucilage. The vegetative plants grow submerged during the rainy season, but are exposed to air when the water level recedes, followed by flowering and setting fruit, dehydrating and eventually dying.

The first Podostemad to be reported was *Mourera fluviatils* by Aublet (1775), in Guiana. Since then, the family has attracted the attention of several generations of botanists. The plants are cosmopolitan in tropical and warm regions, as extending into temperate North East America and East Asia. There are about 48 genera and 270 species worldwide; of which, about 11 genera and 42 species are reported in India, mostly monotypic or oligospecific, reflecting considerable discontinuous variation of morphological characters (Hooker, 1885; Cook, 1996; Mohan Ram & Anita Sehgal, 2001; Mathew, 2003).

The members of the family are commonly called river-weeds with very peculiar vegetative form; revealing many unique morphological, anatomical and ecological features and stands clearly apart from all other angiospermous family (Willis 1902; Schnell 1967; Nagendran et al., 1977; Rutihauser, 1995).

Almost every step in development is unique, with some exclusive features and yet shows a remarkable uniformity in all members even at the specific level. The Podostemaceae are of great morphological and embryological interests because of their great biodiversity in a particular habitat. Kapil (1970b) reported Podostemaceae as an "embryological family" because of several remarkable features such as (a) diverse pattern of female gametophytes development; (b) lack of antipodal cells; (c) absence of double fertilization and endosperm; (d) presence of pseudo embryo sac; (e) suspensor haustoria; (f) lack of plumule and radicle in mature embryo, etc. These characters not only make the Podostemads markedly distinct from other angiosperms, but also biologically interesting and evolutionarily enigmatic.

Yet, from evolutionary perspective the family is enigmatic. Issues ranging from where the origin of the ancestral roots lie within terrestrial groups, to the ecological and biological factors that have resulted in its remarkable radiation into extreme aquatic

environments, remain unexplored. Podostemaceae are given little serious attention in plant biology text. However, the family is untapped resource for ecological and evolutionary study. In fact Podostemaceae may provide valuable means for testing components of the paradigm for slow evolutionary rates and thus small taxonomic sizes that pervades aquatic angiosperms (Arber 1920; Les Donald & Philbrick 1993). Sculpthore (1967) remarked that, "There is surely no stranger and more provocative family of angiosperm than the Podostemaceae".

Although the previous floristic studies of North East India (Haridasan & Rao, 1985; Joseph, 1983; and Balakrishnan, 1983) did not mention anything about the existence of Podostemaceae members in Meghalaya state, India; Kanjilal and Bor (1940) reported only two species of Podostemaceae, that too, they referred from Hooker's Flora of British India (1885). Being one of the two world-renowned hot spots in the world (Swaminathan, 1991; Mohan Ram & Sehgal, 2001), which are of mega-biodiversity centre, practically not much work, in this concern, have been carried out from the North Eastern region of the country. Recently three members of Podostemaceae viz., *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus* Gard. and *Polypleurum wallichii* (R. Br. ex Griff.) Warm. from

Meghalaya, have been reported by Venugopal and Lalruatsanga (2004) along with their possible utilization for crop production.

Therefore, I have chosen *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus* Gard. and *Polypleurum wallichii* (R. Br. ex Griff.) Warm. from Meghalaya, North Eastern India, with particular emphasis on the following objectives:

- To study the morphological aspects of three members of Podostemaceae.
- To work out the embryological characters of the family. This includes various developmental aspects of male and female reproductive organs.
- To study pollen biology, Pollen viability and embryogenesis.
- To monitor the preliminary works on ecology of the three species.

The thesis embodies nine chapters. The first three chapters represent introduction, review of literature and material & methods. Chapter-4 deals with the morphological and embryological aspects of *Hydrobryum griffithii.* The plants are closely appressed to substrate and spreading over stones, more or

less ovoid to circular outline. Leaves filiform- linear, greenish, scattered in groups of 2-3 on the upper surface of the thallus, up to 12 mm long. Fruit capsule, isolobous, 12-ribbed. Flowering starts in late September and fruiting observed in late November.

The flowers of *Hydrobryum griffithii* are bisexual which have two tetrasporangiate anthers. Pollen grains are dyads. The test revealed that 73.68 % of pollen grains were viable in *Hydrobryum griffithii*. The female gametophyte development in H. *Griffithii* is monosporic The development and differentiation of embryo sac in H. *Griffithii* is 5 nucleate, 4 celled structure of Apinagia type B. Nucellar plasmodium begin to development at the two nucleate embryo sac stage. Embryogeny followed the Onagrad type of ontogeny. Endothelium exists up to the differentiation of complete embryo

In chapter-5, aspects of *Polypleurum wallichii* are discussed in detail. The vegetative plant body is fleshy, often branching exogenously and generally free drifting in the flowing water. Thallus attached to substrate at the base only, Ovary sessile, bicarpellary smooth when young, ripening into capsule, 8 ribbed; pedicellate, as long as 5 cm when the fruit gets matured. Flowering starts in early September and fruiting observed in late October; seeds dispersal at the middle of November.

The microspore mother cells are enclosed by a distinct callose Meiosis is of successive type during and after meiosis-II, the pro-orbicules (Ubisch bodies) are released from tapetum and deposited on the pollen exine. The tapetal cells persist until the release of pollen grains in pairs. Pollen grains of *Polypleurum wallichii* occur in dyads, showing 82.60% of pollen viability. Embryo sac development is bisporic; organization conforms to both *Dicraea* type and *Podostemum* type. Nucellar plasmodium formation starts before fertilization. *Polypleurum wallichii* follows solanad type of embryogeny.

Chapter-6 describes morphological and embryological characteristics of *Podostemum subulatus* . The plants is a minute flat, veined, lobulate frond Leaves long and slender, up to 30 mms long, very dense and obscuring the root when viewed from above Fruit a capsule, unequally lobed, 8-ribbed Flowering starts in late august and fruiting observed in early October; seed dispersal at the middle of November.

Each staminal primordium comprises of unilayered dermatogen that covers the multicellular hump-like tissues. Meiosis is of successive type Pollen grains in *Podostemum* also occur in pairs. It is observed that only 36.17 % of pollen grains were viable, which is comparatively low in contrast to the other two species studied. As

soon as the outer integument differentiates on the ovule primordium, the tenuinucellus is demarcated. Nucellar plasmodium develops at the time the megaspore mother cell undergoes meiosis-I. Embryosac is monosporic; an Onagrad type of development, for the first time revealed in *Podostemum subulatus* Gard. and *Hydrobryum griffithii.*

In the chapter-7, the ecological data of the three species are discussed. Different physico-chemical parameters were analyzed and correlated with the ecology of three specimens studied The two study sites having high pH indicates less human activities of different kind. The largest population of the species is observed to occupy an area of full sunlight. It is also established that the three specimens inhabits clean rivers with less pollution and human influences. The family therefore serves as an indicator of clean water. However, physico-chemical analyses indicate that the plants primarily occurred in low nutrient. The rivers in the study site are fast flowing and well oxygenated. Observation shows that the dissolved oxygen is apparently the most essential factor for the successful establishment of these plants, rather than it is the content of other nutrients and the pH. Toxic discharge of effluents from industries and agrochemical residues are serious threats to the Podostemads that have a unique ecological requirement. The last two chapters encompass general discussion and summary & conclusions.

In the present study, two species *Hydrobryum griffithii* (Wall. ex Griff.) Tul., and *Podostemum subulatus* Gard. have been rediscovered and re-described after Hooker (1885), from Meghalaya. Till date, the previous workers mostly confined their studies on female gametophytes only, because of its remarkable reduced nature. The present study, apart from the megasporogenesis and embryogenesis, deals with the details of anther development and microspore formation in all the three members.

Pollen viability was assessed for the fist time in *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemum subulatus* Gard. and *Polypleurum wallichii* (R. Br. ex Griff.). Nutritional aspect of embryo was discussed in the light of suspensor haustorium and presence of endothelium in three species. Though the plant deserve less known economic value, the present work gives an insight into the utilization of these plants as biofertilizers, because of the presence of biofilm of rhizobium cyanobacteria in the holdfast region, for future research.

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Dedicated

to

My Father

2005

NORTH - EASTERN HILL UNIVERSITY

SHILLONG

OCTOBER 2005

DECLARATION

I, *H. Lalruatsanga*, declare that the subject matter of this thesis entitled "Morphological and embryological studies of some Podostemaceae members of North-eastern India" is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University / Institute.

This is being submitted to the North-Eastern Hill University, Shillong for the award of the degree of Doctor of Philosophy in Botany.

(H. LALRUATSANGA)

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Words are inadequate to express my thankfulness to my family members. I dedicated the thesis to my late father Shri H. Kawlkhuma. I have my extreme admiration to my mother Smt Biakveli and sister (Lateii), for their untiring love, patience, determination and highly concern for my study. My acknowledgement is not absolute but for Dr Grace Z, for her support, company and comprehension.

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(H. LALRUATSANGA)

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CHAPTER - 1

Introduction

Podostemaceae Rich. Ex C. Agardh is the largest family of strictly aquatic flowering plants (Philbrick & Novello, 1995; Cook, 1996) and consists of aquatic angiosperms that typically grow on rocks in cascades, waterfalls and rapids where there are great fluctuations in the river water levels. They grow firmly attached to rocks and stones by means of adhesive holdfast or haptera, which secretes mucilage. The vegetative plants grow submerged during the rainy season, but are exposed to air when the water level recedes, followed by flowering and setting fruit, dehydrating and eventually dying.

The plants are cosmopolitan in tropical and warm regions, as extending into temperate North East America and East Asia. Sprague (1933) pointed out that the family should be spelt Podostemaceae and not Podostemonaceae, as is frequently used. The pioneering work on Podostemaceae in India was carried out by Willis (1902 a, b). There are about 48 genera and 270 species worldwide; of which, about 11 genera and 42 species are reported in India, mostly monotypic or oligospecific, reflecting considerable discontinuous variation of morphological characters (Hooker, 1885; Cook, 1996; Mohan Ram & Anita Sehgal, 2001; Mathew,

2003). Out of 42 species, 21 species are endemic, largely confined to Kerela and Karnataka.

The members of the family are commonly called river-weeds with very peculiar vegetative form; revealing many unique morphological, anatomical and ecological features and stands clearly apart from all other angiospermous family (Willis 1902; Schnell 1967; Nagendran et al., 1977; Rutishauser, 1995). The architecture of the plant body deviates remarkably from the root-shoot system common in angiosperms (Rutishauser, 1997, 1999). The plant body has been interpreted as root, stem or a combined shoot (leaf-stem) structure. The family Podostemaceae has been divided into two sub-families, Podostemoideae (43 genera) and Tristichoideae (5 genera) as reported by Cook (1990). The members of Podostemaceae have a plant body (thallus) resembling that of Bryophyte, Phaeophyceae (Algae) and lichens. The adult plants are lacking a clearly defined root and stem system similar to that of other flowering plants. Aerenchyma and vascular tissue are absent. The thallus bears short vegetative shoots on the dorsal margin that are replaced by floral shoots during the reproductive phase. Whether the thallus is of shoot or root origin has been a subject of much discussion till date. The extremely high degree of polymorphism exhibited by many species has made specific delimitations fairly difficult.

In nature flowering commences in September, the flowers are differentiated when the plants are still submerged. Fruit maturation takes place when the water level drops and the rocks become gradually exposed (Mohan Ram & Sehgal, 2001). The Indian Podostemaceae that have been investigated are self-pollinated but cross pollen have also been observed to germinate in petri plate-pollination (Khosla et al., 2000). The ovule number is generally large and seed set high; Vidyashankari (1998a) reported that in *Griffithella hookeriana*, 1.2 million seeds weigh just one gram. The capsule develops rapidly and dehisces to liberate tiny seeds between November and February. The seeds lie in crevices of rocks until next monsoon and germinate with the first shower.

Local peoples in tropical region utilize riverweeds variously. Van Royen (1951) reported new-world species being used as forage for cattle during the dry season. Schultes (1988) has reported that native people of Amazon use certain species for food seasoning. Leaves of a species of *Rhyncholacis are* dried, ~~and~~ pulverized and used as a pepper-like seasoning, while the ashes of burned leaves are utilized as a salt 'substitute' (Schultes, pers. Comm.). In parts of Mexico, species of *Marathrum* are evidently employed as a liver treatment (Novello and Philbrick, 2004). Although sporadic reports exist, there has not been a concerted effort to document the diverse uses of riverweeds. Gessner and

Hammer (1962) reported that the leaves of *Mourera fluviatilis* Aublet are an important food for fishes.

The Podostemaceae are of great morphological and embryological interests because of their great biodiversity in a particular habitat. Kapil (1970b) reported Podostemaceae as an "embryological family" because of several remarkable features such as (a) diverse pattern of female gametophytes development; (b) lack of antipodal cells; (c) absence of double fertilization and endosperm; (d) presence of pseudo embryo sac; (e) suspensor haustoria; (f) lack of plumule and radicle in mature embryo, etc. These characters not only make the Podostemads markedly distinct from other angiosperms, but also biologically interesting and evolutionarily enigmatic.

Almost every step in development is unique, with some exclusive features and yet shows a remarkable uniformity in all members even at the specific level. Xenogamy is common to all angiosperms; a monophyletic origin has been attributed to them (Richards 1986). It is not certain as to whether the absence of double fertilization in Podostemaceae is a mere variation or the angiosperms are polyphyletic in origin. There is no clear-cut answer to this basic question. The unique spatial relationship of the floral parts and synchronization of timing of anther dehiscence and stigma receptivity have ensured selfing within a flower,

although the role of wind cannot be ruled out in affecting pollination. Thus, autogamy prevails whether flowers are chasmogamous or cleistogamous.

The ultimate aim of cleistogamy may be ecologically adaptive characters to avoid the influence of running water and greater reproductive success. Both the autogamy and cleistogamy have led to considerable inbreeding, coupled with high seed set and long-term gene flow. This attribute of Podostemaceae is in direct contrast to other aquatic angiospermous families that have a higher rate of asexual means and consequently have a slow rate of evolution (Philbrick & Novello, 1995). Perhaps, all the reproductive efforts have been invested in seed formation, as vegetative reproduction is almost absent in the family. Reduced types of embryo sac development and a nucellar plasmodium are of particular interest in the family (Battaglia, 1971).

Willis (1915, 1926) believed that the ancestors of Podostemaceae must have been land plants on the bank of rivers that send out creeping roots along the stones of waterfalls. He further hypothesized that they might have got shifted to water at one time and undergone whole of the evolution in water. Rutishauser and Huber (1991) have postulated the step-wise structural changes required to arrive at the present day podostemads from a putative ancestor through certain morphogenetic switches.

However, until we come across living intermediates, the question "Why, how and when did the Podostemaceae settle on rocks, submerged in the running water of the rivers?" posed by Willis (1949) remains unanswered.

Yet, from an evolutionary perspective the family is enigmatic. Issues ranging from where the origin of the ancestral roots lie within terrestrial groups, to the ecological and biological factors that have resulted in its remarkable radiation into extreme aquatic environments, remain unexplored. Podostemaceae are given little serious attention in plant biology text. However, the family is untapped resource for ecological and evolutionary study. In fact Podostemaceae may provide valuable means for testing components of the paradigm for slow evolutionary rates and thus small taxonomic sizes that pervades aquatic angiosperms (Arber 1920; Les Donald & Philbrick 1993; Sculthorpe 1967).

A serious problem associated with the biology of Podostemaceae is the rapid fluctuation of water level in the river, often leaving the plants exposed to bright light. The members are capable of completing the developmental processes from anthesis to full differentiation of embryo within a day under conditions of exposure (Willis, 1902a, b). These facts make it difficult to collect the flowers at the appropriate time. The unusual habitat, combined with peculiarities of life cycle, influences the timing and means by

which collections are made for systematic studies (Philbrick & Novello, 1995). Extremely high percentage of failure in fertilization and the consequent degeneration of the embryo sacs imposed further difficulties in the study of the embryogeny also.

Sculpthorpe (1967) remarked that, "There is surely no stranger and more provocative family of angiosperm than the Podostemaceae". The unique combination of characters presented by this family is unparalleled among the angiosperms, leading to the recent resurgence of worldwide interest. A special volume of Aquatic Botany (1997, vol 57) has been brought out on the Podostemaceae highlighting the current state of the knowledge and scope for future work. Dahlgren (1980) placed Podostemaceae in super order Podostemiflorae, commenting that "...they are so specialized that they cannot be associated with any other super order without severe reservation." An extreme view has been taken by Cusset and Cusset (1988b,c), who proposed the Podostemopsida as a class of angiosperms, equivalent in rank to monocotyledons and dicotyledons. Thus, even after more than a century and a half, the relationships of riverweeds remain unresolved at nearly every higher level of classification.

The family is also noted for its high degree of speciation, with a number of species and some genera being endemic to a single group of rapids or waterfalls (Philbrick & Novello, 1995).

However, it is very difficult to interpret patterns of species endemism when taxonomy is unreliable.

The Podostemaceae thus offer a challenging problem to the morphologists, the anatomists, the embryologists, the ecologists, and the physiologists through the problems associated with various aspects of their biology. All investigations so far, deal chiefly with the external morphology, megasporangium and female gametophytes. Very little information is available on microsporangium, details of microsporogenesis, and development of male gametophytes and embryo development in the members of Podostemaceae.

~~Although~~ The previous floristic studies of North East India (Haridasan & Rao, 1985; Joseph, 1983; and Balakrishnan, 1983) did not mention anything about the existence of Podostemaceae members in Meghalaya state, India; Kanjilal and Bor (1940) reported only two species of Podostemaceae, that too, they referred from Hooker's Flora of British India (1885). Being one of the two world-renowned hot spots in the world (Swaminathan, 1991; Mohan Ram & Sehgal, 2001), ~~which are~~ and one of the world's mega-biodiversity centres, practically not much work, in this concern, have been carried out from the North Eastern region of the country. Recently three members of Podostemaceae viz., *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus*

Gard. and *Polypleurum wallichii* (R. Br. ex Griff.) Warm. from Meghalaya, have been reported by Venugopal and Lalruatsanga (2004) along with their possible utilization for crop production. Consequently, the present study deals with the morphological and embryological aspects of three members of Podostemaceae, viz. *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus* Gard. and *Polypleurum wallichii* (R. Br. ex Griff.) Warm. from Meghalaya, North Eastern India.

The thesis embodied particular emphasis on the following objectives:

- To study the morphological aspects of three members of Podostemaceae, viz. *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus* Gard. and *Polypleurum wallichii* (R. Br. ex Griff.) Warm.
- To work out the embryological characters of the family. This includes various developmental aspects of male and female reproductive organs.
- To study pollen biology, Pollen viability and embryogenesis.
- To ~~monitor the~~ preliminary works on ecology of the family.

CHAPTER – 2

Review of Literature

The first Podostemaceae to be reported was *Mourera fluviatils* by Aublet (1775), in Guiana. Since then, the family has attracted the attention of several generations of botanists. Tulasne (1852) made an invaluable contribution to the morphology and taxonomy of the Podostemaceae through his celebrated work "Monographia Podostemacerum" which is remarkable for the beautiful illustrations, combining both technical accuracy and artistic perfection. He made a systematic treatment of various genera known to his day and tried to draw proper demarcation between genera and species. He also suggested that the thallus of the Podostemaceae is morphologically a flattened photosynthetic root which gives rise to floriferous secondary shoots. This view was accepted by Willis (1902,a, b) and Engler (1930).

Willis (1902a,b, 1914, 1915, 1926) studied Indian (including Srilankan) Podostemaceae in their natural habitat, directing major attention to their morphology, taxonomy, ecology and phylogeny. Earlier, Gomez (1828; cited from Willis 1902a) has described *Podostemum wallichii* R. Br. from India. Weddell's (1873; cited from Willis, 1902a) monograph continued to be the standard in the listing and description of the Indian species until

the publication of Willis's (1902a) most important paper entitled "A revision of the Podostemaceae of India and Ceylon".

It was John Lindley (1830), who for the first time suggests the Podostemaceae were, in fact, dicotyledonous plants. The general assumption prior to that was that they occupied some nebulous position around the Naiadaceae, Juncaginaceae or even Lemnaceae, if they were even angiospermous. Bentham and Hooker (1880) placed the family in a separate series called Multiovulatae Aquaticae in his Monochlamydae. His Podostemaceae included Tristichaceae and Hydrostachyaceae. Although it was previously placed together with Crassulaceae, Saxifragaceae and Hydrostachyaceae in Rosales (Warming, 1891; Engler & Prantl, 1930), Hutchinson (1926, 1973) grouped them with Hydrostachyaceae in the order Podostemales. As Takhtajan (1966) pointed out, it was Warming in 1891 who, for the first time suggested a connection with Crassulaceae, and additional data have continued to support this opinion. Hydrostachys was subsequently removed to its own family, and they along with Callitrichaceae, moved to the Asteridae.

Willis (1915a) expressed his approval of Warming's (1891) separation of Hydrostachys from Podostemaceae to a separate family Hydrostachyaceae. Simultaneously he assigned *Tristicha, Terniola* (=*Lawia*) and *Weddelina* into a new family-Tristichaceae

to be kept apart from the Eupodostemaceae. Willis (1915b, 1926a) in his subsequent papers, proposed that the Tristichaceae as well as the Eupodostemaceae are phylogenetic descendants of some unknown land plants which suddenly took to an aquatic habitat due to an 'adaptive' mutation(s). It may be added that Willis (1926b) has used much of his evidence from his studies on the Podostemaceae in support of his "Age and Area hypothesis". Van Royen (1951) and later Cook (1990) proposed the recognition of two subfamilies, the less specialized Tristichoideae Engl. with about six species in four genera arranged in two tribes (Tristicheae Tul. and Weddellineae Wedd.), and the more advanced subfamily Podostemoideae Wedd. with the remaining genera and species. The latter subfamily is also subdivided into two tribes, Mourereae Benth. & Hook.f. and Podostemeae Dumort. Willis (1914) and more recently, Cusset and Cusset (1988) have proposed Tristichaceae as a segregate family.

However, Kita and Kato (2001) conducted phylogenetic analyses of 20 genera and 30 species of Podostemaceae based on *Mat* K gene sequence data. Their results support the recognition of either two or three subfamilies in Podostemaceae. Their analyses indicate two major clades in the family. The smaller of the two included taxa (e.g., *Dalzellia, Indotristicha, Tristicha*) that are presently recognized in subfamily Tristichoideae. The larger clade comprised all taxa placed in subfamily

Podostemoideae, along with *Weddelina squamulosa* Tul., which some authors (e.g., Engler 1930) recognize as distinct subfamily (Weddelinoideae).

The placement of the family in a modern system varies slightly. Dahlgren (1989), in his last revision, placed the taxon in the Saxifragales next to Crassulaceae. Thorne (1992) retains the order, but positions it next to his Saxifragales in the Rosanae. Takhtajan (1966) has Halograles, Podostemales and the Gunnerales as the final three orders in his Saxifraganae. Recent molecular analyses have suggested that this family is a close relative of Clusiaceae in the Malphigiales (Savolainen et al., 2000; Soltis et al., 2000; Gustafsson et al., 2002). The unique embryological features like binucleate glandular tapetum, anatropous tenuinucellate ovules, formation of pseudo-embryo sac, absence of triple fusion and consequently absence of endosperm, tegmic seeds, etc. justify to give the rank of an independent order Podostemales with a single family Podostemaceae.

Podostemaceae vary enormously in morphology and often show a thalloid vegetative body due to the dorsiventrally flattening of root, shoots (stem) or a combination of the two (Rutishauser, 1997). Leaves may be complex and upto 2 metres long (*Mourera*) or moss like, composed of green scales that are only one cell

layer thick (*Tristicha* and its allies). Indeed, how to best interpret the fundamental components (roots, stems, leaves) of the vegetative plant body of Podostemaceae is controversial. Willis (1902 a) has reviewed the previous work on Indian Podostemaceae and discussed exhaustively in detail the various morphological problems posed by different authors. He accepted and produced evidence for Tulasne's (1952) view that the thallus in Podostemaceae is morphologically a root. According to him, *Terniola* (=*Lawia*) is an exception in having a thallus, which is morphologically a shoot. He has also a comprehensive list of Asiatic species published in another paper (Willis 1902b), further elaborated many of his views and presented information on the ecology of these plants. The perplexing and diverse morphology of the Podostemaceae prompted Willis to disbelieve that Darwinian Natural Selection could provide an explanation for this existed within what he considered to be uniform conditions of rapidly flowing water in the warm tropics. In his opinion many of the characters were 'unadapted'; he proposed that large mutations rather than accumulation of small ones accounted for the diversity observed (see Mabberley 1997).

Rutihauser and co-workers (Rutihauser 1995, 1997; Rutihauser & Huber 1991) applied the concept of "fuzzy morphology" concluding that the structural categories of root,

shoot and leaf in Podostemaceae do not necessarily imply correspondence to the classical root-shoot model of angiosperms.

Another view was proposed by Jager-Zurn (1997) who used the "Principles of variable proportions" to apply classical morphological interpretations to the same structures, thus implying that the vegetative construction in the family does not differ from that of other dicotyledons.

An additional approach uses neutral terms (e.g. Thallus) as purely descriptive expressions (Sculpthore 1967; Nagendran 1983; Mohan Ram & Sehgal 1992, 1997); however fails to establish a comparative framework useful for interpreting the vegetative morphology of Podostemaceae relative to other angiosperms.

These rather disparate views emphasize the remarkable degree to which the vegetative morphology has been modified in this large aquatic plant family. The proposal by Cusset & Cusset (1988a,b) that Podostemaceae belong to their own class of angiosperms (Podostemopsida) is one of the consequences of the difficulties in interpreting the vegetative form of these odd plants.

Cusset (1972, 1987, 1992) and Cusset & Cusset (1988a, b, 1989) have discussed the structural details, taxonomic delimitations, geographic variations and evolution of various members of Podostemaceae. Cusset's description of several taxa

such as *Hydrobryopsis sessilis, Zeylandium sp., Griffithella hookeriana* and other species are based on Indian collection. Jager-Zurn (1992, 1995, 1997) in a series of paper has made a serious effort in understanding nature of the thalloid plant body of Podostemaceae. Again, Rutihauser and Huber (1991) have given a comprehensive account of developmental morphology of *Indotristicha ramossisima* collected from Western Ghat of India.

Although Podostemaceae occupy ecologically extreme environments, they are one of the most successful of aquatic plant group, given their taxonomic size. Different species of Podostemaceae often co-occur, although their ecological affinities are unclear. For example, Willis (1902, p. 275) reported that in Ceylon and India 'each species appears to affect a particular class of habitat'. Cook (1996) corroborated Willis's observation that different taxa often grow together.

Our knowledge on the embryology of the family as a whole is limited. Previous investigators like Went (1908, 1910, 1912, 1926b), Magnus (1913), Chiarugi (1933), and Razi (1949,1955) confined their attention mainly to the development of ovules and female gametophytes. Mukkada (1959, 1962 b) investigated the embryology of three Indian Podostemaceae and traced the development of male and female gametophytes and embryo. Went (1908, 1910, 1912, 1926b) studied the female gametophyte in

Apinagia, Cladopus, Oenone, Lophogyne, Mourera, Rhyncolacis and Tristicha. He found that in all these genera, the embryo sac is a reduced bisporic type. The functional dyad nucleus divides to form two nuclei, of which the chalazal degenerates very early or may remain as an inert constituent of the developing embryo sac. The micropylar nucleus divides twice to give rise to four nuclei that organize into an egg apparatus and a single upper-polar nucleus. The mature embryo sac is thus 5-nucleate or 4-nucleate depending upon whether the primary chalazal nucleus persists in the embryo sac or has disintegrated completely. This mode of development was confirmed in *Podostemum ceratophyllum* (Hammond, 1937) and in *Terniola zeylanica* (=*Lawia zeylanica*) and *Griffithella hookeriana* (Razi, 1949).

However, Magnus (1913) described an even more reduced embryo sac, the so-called 'Podostemum' type, with only four nuclei, in *Podostemum subulatus, Hydrobryum (=Zeylandium) olivaceum* and *Farmeria metzgerioides*. Chiarugi (1933) reported the same structure in *Weddelina squamulosa*. According to these authors the nucleus of the functional dyad cell undergoes just two divisions giving rise to a 4-celled embryo sac comprising two synergids, an egg and one cell containing the polar nucleus. Magnus (1913) reported a still more peculiar embryo sac in *Dicraea elongata*, wherein, even the first division of functional dyad nucleus is followed by wall formation. The two cells divide

again forming four cells, which constitute a single synergid, an egg and two antipodal cells. Thus, the embryo sac is tetra-nucleate but the polar nucleus is absent.

Although the studies of Went (1908, 1910, 1912, 1926) were taken for granted, subsequent work of Magnus (1913) was not entirely accepted to embryologists like Schnarf (1931,1936), Maheshwari (1955), who, that time and again, have expressed that a reinvestigation of the species studied by Magnus (1913) was desirable. Razi's (1949, 1955) contribution to the embryology of Podostemaceae also met with acute criticisms from Mukkada (1962a, b, 1964, 1969), Chopra and Mukkada (1966), Jager-Zurn (1967), Battaglia (1971), and Nagendran (1974).

The development of female gametophytes in Podostemaceae is of particular interest because all the types are exclusive to this family investigated so far (Battaglia, 1971). Again, the nutrition of embryo sac is an intriguing problem associated with the embryology of Podostemaceae. Magnus (1913) reviewed that, in a normal angiosperm, the antipodal cells are concerned with the nutrition of embryo sac and ultimately, of the zygote.

Mukkada (1962b, 1963, 1964) reported that in Podostemaceae, *Dicraea* alone possesses antipodal cells; while there is a single nucleus in the rest of the members. Occasionally,

the degenerating primary chalazal nucleus may also persists. As in *Terniola* (Razi, 1949, 1955) and *Zeylandium* (Razi, 1955), the nucleus of the micropylar dyad cell may also divide but usually it degenerates before division. It is also surprising to see the report of Arekal and Nagendran (1975a,b, 1977a,b), Nagendran and Arekal (1976) and Kapil and Bhatnagar (1975) that antipodal cell is absent in Podostemaceae. Went (1908, 1910, 1912, 1926), Magnus (1913), Chiarugi (1933), Razi (1949), Mukkada (1962a,b, 1964, 1969) and Chopra and Mukkada (1966) reported that neither endosperm nor primary endosperm nucleus is found due to the absence of triple fusion in all the members of Podostemaceae investigated so far.

Went (1908) observed that in all the species he studied, the outer integument arises first and forms the micropyle; Hammond (1937), Magnus (1913) and Razi (1949) confirmed that the inner integument differentiates later; however, Mukkada (1962) observed in *Terniola* that both the integuments are initiated simultaneously.

Battaglia (1971) studied the development of the male and female gametophytes, the nucellar plasmodium, embryogenesis and seed structure in *Weddellina squamulosa* Tul. A reinvestigation of embryo sac development reveals a five-nucleate '*Apinagia*' type of embryo sac. The embryo sac is monosporic in

origin. The description of a bisporic embryo sac development (*Podostemum* type) in *W. squamulosa* Tul. by Chiarugi (1933) is refuted. The structure of the gynoecium in *W. squamulosa* Tul. differs from other Podostemaceae. The bicarpellate ovary consists of four parts. The main part, above an ascidiate region is the syncarpous zone with an axile placenta, superimposed on this is a paracarpous region that lacks a septum. The uppermost section develops an additional 'apical septum', a mode structurally connected with anacrostyly. This brings about a slender (compound) style (instead of stylodes as in other Podostemaceae) and a globular stigma. The embryo sac is bisporic and the chalazal dyad cell is functional (Maheswari Devi et al. 1995).

According to Battaglia (1971) two types of embryo sac development occur: Apinagia type and Dicraea (*Polypleurum*) type. However, observation by Arekal and Nagendran (1975b) on *Hydrobryopsis sessilis* shows the presence of *Podostemum* type of embryo sac. Arekal and Nagendran (1976) had also suggested *Willisia* type of embryo sac development.

Very little has been published on the microsporangium, development of male gametophyte, embryogeny, seed coat and pericarp (Mukkada 1969). Previous workers neglected ontogenic studies on the microsporangium. Razi (1949) reported a four-layered anther wall in *Griffithella hookeriana* where the tapetum is

secretory and the cells remain uninucleate. Mukkada (1962) observed 5-layered anther wall in *Dicraea*, being the tapetal cells remain uninucleate; while in *Indotristicha* and *Terniola*, the anther wall is 4-layered and the tapetal cells become binucleate. Meiotic divisions are said to be of the successive type in Podostemaceae (Maheswari, 1950; Subramanyam, 1962). But, Mukkada (1962) have shown that *Dicraea* with double pollen grain follows successive type while *Indotristicha* and *Terniola* with individual pollen grain exhibit simultaneous type. The pollen grains are shed individually/monads (Tristichoideae) or in pairs/dyads (Podostemoideae). A tetrad of microspores is formed as a result of simultaneous cytokinesis in Tristichoideae and successive cytokinesis in Podostemoideae (Mohan Ram & Sehgal, 2001).

Palynological studies on the family conducted by Nair (1965), Vartak and Kumbhojkar (1984) revealed that capsule develops rapidly and dehisces to liberate tiny seeds between November and February. The seeds lie on rocks until next monsoon and germinate with the first shower. Podostemaceae species typically flower and set seeds in abundance, which is unusual for submerged aquatic plants (Sculthore, 1967; Philbrick & Novello, 1995, 1997; Philbrick & Les, 1996). The ovule number is generally large and seed set high; Vidyashankari (1998 a) reported that in *G. hookeriana*, 1.2 million seeds weigh one gram. Khosla and Mohan Ram (1993) reported in *Polypleurum stylosum*

that each flower produces about 3100 $^{+}_{-}$400 pairs of echinate pollen grains; seeds are non-endospermous and minute, 1gm weight contains about 700, 000 seeds.

During blooming period, a large number of pollinating insects visit the plant. Rutishauser (1997) reported that most Podostemaceae are wind-pollinated or autogamous. A few Neotropical genera such as *Mourera* (Podostemoideae) and *Weddelina* (Tristichoideaea) show polyandry, probably as an adaptation to insect-pollination. Both biotic (insect) and abiotic (wind) pollination have been reported, but little empirical data are available to support specific claims. There is a very little chance for hydrophilly since the flowers open above the level of water. It is skeptical to see the report of Hall (1971) that an African species *Polypleurum submersum* J.B. Hall is pollinated by pollen, transported across the water surface. Generally, the pollen grains mature by the time the ovules are at the archesporial stage (Chopra & Mukkada, 1966).

The entry of pollen tube is porogamous and syngamy occurs in a normal manner. However, the fate of second male gamete is unknown. Khosla et al., (2001) reported in *Griffithella hookeriana* that a large number of pollen grains germinate on the stigma and the pollen tubes enter the ovules 16-18 hrs after pollination. It has been clearly demonstrated that two male gametes are formed

(Mukkada, 1962a, b; Chopra & Mukkada, 1966; Mukkada & Chopra, 1973). Went (1908) reported that the pollen tube enters through the micropyle and fuses with the synergid. Later, the cytoplasm of the synergid and the male nucleus discharged in it, passed into the egg cell. Razi (1949) observed the fusion of the two male gametes -one with the egg and another with the polar nucleus, thus confirming double fertilization. Contradictory to these observations, Mukkada's (1962b) work proved that the syngamy is normal but the second male gamete is not discharged from the pollen tube; or, if discharged, may not reach and fuse with the polar nucleus. A small nucleus with a prominent nucleolus is often seen in the persisting tip of the pollen tube, which is presumably the undischarged second male gamete. Thus, the fate of second male gamete has not been unequivocally ascertained. Whether or not the second male gamete degenerates prior to or after entering the synergid is also not known. In *Polypleurum stylosum*, fruits appear mature in an incredibly short period of 4-5 days after pollination (Khosla, et al., 2000).

An exclusive feature of Podostemaceae is the presence of large pseudo-embryo sac. The nucellar cells situated below the megaspore mother cell enlarge considerably, especially in the longitudinal direction. Their walls become extremely thin and stain lightly that they are likely to be overlooked in most preparations. This eventually results in large cavity or pseudo-

embryo sac, which contains numerous nuclei. In all Podostermaceae, the pseudo-embryo sac is a provision to receive the growing embryo, which finally fills the entire space. Chopra and Mukkada (1966) reported in *Indotristicha* that the pseudo-embryo sac is cut off from the neighboring tissues by the thick inner wall of the inner integument and provides an 'ideal water reservoir' (see Arber, 1920). Arekal and Nagendran (1975a) suggested the term nucellar plasmodium in place of pseudo-embryo sac, which is etymologically more precise since organization of a multinucleate protoplast form nucellar cells situated below the developing embryo sac.

Mukkada (1969) observed that the nucellar cells remain intact upto the time of fertilization with healthy nuclei and vacuolated cytoplasms in *Terniola zeylanica*. The disintegration of cells start from micropylar end and proceeds basipetally. In *Dicraea stylosa* and a number of other members (Mukkada, 1962a, 1964; Magnus, 1913; Razi, 1949, 1955), the pseudoembryosac is fully formed much before the embryo sac is organized, whereas its formation in *Indotristicha ramossisima* (Chopra & Mukkada, 1966) and *Terniola zeylanica* (Mukkada, 1969) is a post-fertilization phenomenon. Most of the workers observed a striking parallel feature between Podostemaceae and Orchidaceae in that members of both families show the formation of suspensor haustoria through which the developing embryo derives nutrition

directly from the maternal tissues, thus, compensating for the absence of endosperm. The haustorium in *Indotristicha* is large, unbranched multinucleate structure as was observed in *Terniola* (Mukkada, 1966) and differs from that in *Dicraea* (Mukkada, 1962a) where it extends into several tubular haustorial branches.

Embryogeny is of solanad type. The zygote passes through the conventional quadrant, octant, heart and torpedo stages and eventually forms a mature embryo with two cotyledons and a radicular pole, but plumule failed to differentiate (Mohan Ram & Sehgal, 2001). But Nagendran et al. (1981) mentioned that hypophysis and epiphysis - the indicators of the two poles are recognizable only on the topographical grounds. Mukkada (1969) and Mukkada and Chopra (1973) have noticed a prominent plumule and conical stem tip in *Dicraea (Terniola), zeylanica* and *I. ramossisima* respectively.

Podostemaceae produce capsular fruits. Species of sub-family podostemoideae have two-valved capsule; although valve behavior (e.g. one shed or not) varies among taxa. Despite the reports of some authors (Went, 1929; Accorsi, 1944) that capsules maturing within 24 hours of anthesis, the genera like *Apinagia*, *Oserya*, *Marathrum*, *Mourera*, *Podostemum* and *Vanroyenella* capsule may appear mature within few days of flowering; seed maturation is not complete until two or three weeks after anthesis

(Grubert 1974; Philbrick & Novello 1995, 1998; Oropeza et al., 1998, 2002). Murguia-Sanchez et al., (2001) proposed that the seeds mature at the expense of carbohydrate stored ~~in the~~ starch filled placenta and ovary wall. By the time flowering has occurred, the vegetative parts of the plant often have become dried and senesced. Consequently, photosynthate would need to be mobilized from stored sources quickly to support seed maturation.

Seed production is common in most species ~~and no other mechanisms of pollen transfer are apparent~~. Flowering and seed production is generally prolific in Podostemaceae. Capsule dehiscence leads to the release of tiny (<0.5 mm long), dry seeds. Grubert (1974) documented a seed production per plant ranging from 2,360 for *Apinagia multibranchiata* (Mathiensen) Royen to 1,020,000 for *Mourera flavatilis* Aubl. These examples illustrate the high potential for sexual reproduction in the family; Podostemaceae are typically highly sexual plants.

Mature seeds of all Podostemaceae possesses an outer integument composed of enlarged mucilaginous cells that are collapsed when dry, but quickly becomes sticky when wetted (Grubert 1970, 1974, 1976; Philbrick, 1984). The outer integument seems to play an important role in the initial attachment of seeds to the substratum. When wet seeds are allowed to dry on a rock, they become attached firmly and are not dislodged when the rock

is again wetted. Successful recruitment requires that seed attachment be followed by seedling establishment. Phenology of seed germination in nature is not known; although it likely occurs when water levels are high.

Our cytological knowledge of the Podostemaceae is highly inadequate. One of the major reasons for very little cytological work in this family is the absence of roots. The only earlier report available in the literature is that of Magnus (1913) on *Dalzellia zeylanica* (2n=20). Karyotype analysis was performed using thallus tips in three taxa of the family Podostemaceae by Uniyal and Mohan Ram (1994). The chromosome numbers for *Polypleurum stylosum* (Wight) Hall (2*n*=34) and *Hydrobryopsis sessilis* (Willis) Engler (2*n*=26) have been reported. The chromosome number (2*n*=30) in *Dalzellia zeylanica* (Gardner) Wight differs from the previous count (2*n*=20) as is reported by Magnus (1913). Critical notes have been made on the karyotype characteristics and their interrelationship among species.

Philbrick and Novello (1995) analyzed the karyotype of three Podostemaceae genera for the first time. Somatic chromosome numbers were 2*n*=28 (*n*=14) for *Oserya coulteriana* Tul. and *Vanroyenella plumose* (subfamily Podostemoideae). Their karyotypes were similar (13*n*+1 s*n* chromosome pairs). The pantropical species *Tristicha trifaria* (Bory ex Willdenow) *Sprengel*

(subfamily Tristichoideae), with $2n$=20 (n=10), had a karyotype of 10 n chromosome pairs. Although the karyotypes were homogeneous in the species studied, intergeneric variation of their genomes was evident in chromosome size and genome length. The largest chromosomes and genome lengths were found in *O. coulteriana*, while chromosomes of intermediate size occurred in *V. plumose*; *Tristicha trifaria* had the smallest chromosomes. New data presented herein, combined with data from the published literature, indicates that Tristichoideae is uniform with x=10 and that polyploidy is the principal mechanism of chromosome evolution. In contrast, in the Podostemoideae the main mechanism of evolution is aneuploidy. Podostemoideae possess a range of base numbers: n=10, 13–15 and 17. Diploidy may be the underlying reason for such variation. Cytotaxonomic evidence supports the recognition of Podostemaceae and Tristichaceae as distinct families.

Stebbins (1971) predicted that increased karyotype asymmetry is associated with increased morphological specialization. Morphological specialization, however, is somewhat of norm in Podostemaceae, a family characterized by remarkable morphological modification relative to most other angiosperms (e.g. Rutihauser, 1997). If karyotype asymmetry were used as an indicator of specialization, one would predict that Asian Podostemoideae (with higher karyotype asymmetry) would have

more specialized morphologies than the American members of the same sub family.

Mohan Ram and Sehgal (1998) focused special attention on the sequence of developmental events from seed to flowering in *Indotristicha ramosissima* (Wight) Van Royen (Tristichoideae), the only Indian species that seems to correspond to the Classical Root-Shoot (CRS) model. In *I. ramosissima,* exogenous root primordia arise from the flattened radicle, of which only three develop into main roots with asymmetric root caps. Although the mature seed lacks a plumule, a 'primary axis' of limited growth is formed at the apex. The plant body, with its numerous long branches, develops from the main roots.

The unique preference of the members for a soil -less, rocky or gravelly substratum in well-aerated running water led Went (1926a) to conclude that no species can be grown artificially. However, Hammond (1937) succeeded in growing *Podostemum ceratophyllum* under artificial conditions. Flowering under in vitro condition has been recorded for the first time in *P.stylosum* (Sehgal et al., 1993) and *I. ramosissima* (Mohan Ram &Sehgal, 1997). Vidyashankari's (1988a) studies on *Griffithella hookeriana,* is the first exhaustive account on the developmental biology of Indian Podostemaceae.

Comparisons are made of early developmental patterns among the seedling of nine Asian and Australian species of Podostemaceae. Special attention is focused on determining at what stage the ontogeny of the root-shoot versus thalloid growth forms diverge. One to several leaves occupies the shoot apical area in species with endogenous adventitious roots, while no leaves are formed in species with exogenous root (Suzuki, et al., 2002). Flowering has been observed to be stimulated by subjecting plants to water and nutrient stresses. A model of seedling development is presented that depicts interspecific variability among species examined. The homology of the thallus in Podostemaceae remains unclear (Mohan Ram &Sehgal, 1997).

The Podostemaceae is particularly noteworthy for its many monotypic genera, and a large number of narrowly endemic species; for instance with several forms restricted to different stretches of a single river; such situations are located in tropical South America, Madagascar, Sri Lanka, India, Myanmar, and Indonesia (Sculthorpe, 1967). A large number of species have been spread by intentional or accidental human introductions to areas beyond their native range, and in many cases have occupied vast areas and had serious ecological impacts.

Riverweeds are confined ecologically to river rapid habitats and their high global diversity evades explanation. Phylogenetic

trees constructed from *rbc*L sequence data reveal an unusual biogeographic pattern. The basal clade (subfamily Tristichoideae; = Tristichaceae) represents a Gondwana floristic element with *Tristicha* (Australia, Madagascar, Africa, tropical America) distributed pantropically and other genera exhibiting radiations in India and south/southeast Asia. The basal taxa of a second clade (subfamily Podostemoideae; = Podostemaceae sensu stricto) are uniformly South/Central American in distribution (e.g. *Apinagia, Marathrum, Mourera, Oserya, Vanroyenella*). Following in phylogenetic order are *Podostemum* (South/North America), *Cladopus* (Japan, southeast Asia), *Zeylanidium, Polypleurum* and *Farmeria* (southeast Asia and India). The biogeographical affinities of riverweed genera indicate that diversification in this group has experienced both primary (Tristichoideae) and secondary (*Zeylanidium, Polypleurum, Farmeria*) tropical radiations. Support for the molecular phylogeny (which adds credibility to the biogeographical patterns observed) is provided by various morphological features such as the presence of an andropodium and pollen dyads, which are synapomorphic for the clade that contains *Podostemum ceratophyllum* (North America) and four Asian Podostemoideae. The migration of riverweeds into Laurasian floristic provinces and their subsequent return to tropical environments is presented as one hypothesis to explain

their diversification. A similar phytogeographical pattern occurs in the diverse, aquatic (and arguably related) family Haloragaceae.

Most of the Tropical Rivers represent the most heavily polluted and often causes severe damage to the environment. Podostemaceae often represent the dominant submerged vegetation in tropical rivers, especially in river-rapid and waterfall habitats. Anecdotal accounts implicate water pollution as detrimental to populations of Podostemaceae (Augustin et el., 1997). This paper examines chemical variables and their relationship to the occurrence of species of Podostemaceae. Twenty-eight tropical rivers in which Podostemaceae occur were sampled in the Mexican states of Nayarit, Jalisco, Guerrero and Oaxaca on the Pacific slope, and Veracruz and Oaxaca on the Atlantic slope. Assays were conducted for total nitrogen, total phosphorus, ammonia, nitrates, orthophosphates, major cations (Na, K, Mg, Ca), temperature and pH. Seventeen rivers had nutrient levels below the level of detection; Podostemaceae occurred primarily in low nutrient (oligotrophic) rivers. Six rivers had detectable levels of total nitrogen (160–2050 µg/ l) and total phosphorus (100–720 µg/ l). Five additional rivers had detectable levels of total nitrogen, but lacked phosphorus. Sodium was the dominant cation in all but one river. These results indicate that some species of Podostemaceae can tolerate eutrophic conditions,

at least during the period of low water. Studies of ambient water chemistry throughout the year thus become a necessity.

The presence of Podostemaceae, either seed or entire plant body, was also observed in Amazonian fish stomachs (Santos et al., 1997; Santos & Rosa, 1998), but had not yet been mentioned for Atlantic Forest fishes. The plants are typically found in lotic environments, on sandy and rocky substrates being associated to coarse litter from the riparian forest, roots, algae and a wide variety of insects. Podostemaceae thus function as an autochthonous food source for fish and aquatic invertebrates, as well as a habitat for larval forms of many insect groups. Philbrick and Novelo (1997) stated that little is known about Podostemaceae seed dispersion by biological vectors, so that the presence of Podostemaceae in the stomachs of *Astyanax* could have connections with the biology of these plants.

Though the habitat is not rare, it is particularly susceptible to human impacts. Dam building, pollution and siltation adversely affect Podostemaceae populations. Hydroelectric dam and power project construction, which at the same time changes the seasonal cycle of high and low water and convert natural lotic system to managed lentic system, is responsible for the loss of Podostemaceae from many localities in Argentina, Brazil, Uruguay and South India also.

Detrimental influence also results from chemical and physical pollution. Nutrient loading is associated with epiphytic algal growths that cover plant surfaces and predictably reduces their productivity, which in turn affects reproduction. Silt is also detrimental because it covers solid substrata and plant surfaces, and reduces the ability of seeds to attach. Water-borne sediment could also scour seeds, seedlings and mature plants from their places of attachment. Sometimes, obvious nutrient pollution and siltation were associated with reduced population vigor in species of *Apinagia, Marathrum, Oserya, Mourera and Podostemum* as reported by Philbrick and Novello (2004).

Podostemaceae are subjected to a wide range of anthropogenic disturbances. Toxic discharge of effluents from industries and agrochemical residues are serious threats to the Podostemaceae that have a unique ecological requirement. Cross Bell (1990) has studied the effect of effluents of rubber factory in Kanyakumari district in Tamil Nadu, India, and has observed the elimination of three species of Podostemaceae in the down stream due to acidic discharges. Predictions regarding global warming may also adversely affect Podostemaceae as they occur in seasonally pulsating rivers (Grimm 1993, Philbrick 1997). A continuous and consistent effort on the part of scientists is required to evolve methods of *ex situ* and *in situ* conservation.

CHAPTER – 3

Materials and methods

3.1 Development of flowers

Flowers of *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus* Gard and *Polypleurum wallichii* (R. Br. ex Griff.) Warm., at various stages were collected from Janiaw locality, Mawsynram (91° 50'N – 25° 20'E) and Umtienger locality (91° 70'N – 25° 40'E) respectively, East Khasi Hills, Meghalaya state, India (Plate 3).

Formalin acetic-alcohol (FAA) and Carnoy's fluid were used as the fixative and the materials were later transferred to 70 % alcohol. Since several species of the Podostemaceae grow together, the scraping of plants from the rocks invariably gives a mixed collection. Often the floral characters differ only in minor points and hence utmost care has to be exercised in sorting the collections. Since detached flowers are difficult to identify, it is preferable to reject them and to sort out only thalli with attached flowers, to avoid errors due to mixing up of materials.

The materials were prepared for microtomy by the usual methods of dehydration in the Tertiary butyl alcohol series followed by impregnation with paraffin wax (Johansen, 1940). For the study of the female gametophyte and the development of the

embryo, entire ovaries were sectioned longitudinally at 7-12 microns. Transverse sections at 10-12 microns proved most suitable for the development of the microsporangium and the details of microsporogenesis. The ovules, being extremely minute, are unsuitable for dissections, but whole mounts of embryos could be made by tapping the seeds in a drop of acetocarmine under a cover glass. For the light microscopy the materials were fixed in a phosphate buffered solution of 2-3% glutaraldehyde, dehydrated in propanol and embedded in glycol methacrylate (Technovit 7100). To ensure proper fixation, the air was removed before fixing by vacuum pump.

A rotary microtome was used to produce 7-10 µm thick sections that were stained with safranin-fastgreen and erythrosine. Callose was stained with decolorized aniline blue (Heslop-Harrison, 1968b; Shivanna and Rangaswamy, 1993). The sections of material were brought down in a series of alcohol and to Distilled water. A drop of decolorized aniline blue was poured on the sections over the slide, covered with black paper and kept overnight. Proteins were stained with mercuric bromophenol blue (Mazia, et al., 1953) and nucleic acid with Azure blue (Heslop-Harrison, 1979). Photomicrographs were taken by using Nikon E600 and Leitz Wetzlar Germany (Type 307-083. 103) fluorescence microscope.

3.2 Scanning Electron Microscopy

External surfaces of the plant body, androecia and gynoecia were studied morphologically by using Scanning electron microscope (SEM). The following methods were employed for SEM studies:

1). Flowers were collected, androecium as well as gynoecial parts were dissected longitudinally with razor blades, fixed in 2-3% glutaral dehyde prepared in 0.1M phosphate buffer, pH 7.2 at 4°C for 8 hours, thoroughly washed in 0.1M phosphate buffer and post fixed in 1% OsO_4 for 2 hours.

2). The materials were then dehydrated in increasing concentration of acetone.

3). Dehydrated materials were critical point dried in a Tetramethylsilane (TMS) solution.

4). Dried materials were dissected with razor blades, sputter coated with gold in an Eiko ion sputter, JFC-1100.

5). The materials were examined in Joel (JSM-6360) scanning electron microscope.

3.3 Pollen Viability

Pollen viability of fresh pollen grains was tested by using Fluorochromatic Reaction (FCR) Test (Heslop-Harrison, 1970; Shivanna and Rangaswamy, 1993).

1). Stock solution of Fluorescien diacetate solution (FDA) was prepared in acetone (2mg/ml). It can be stored in the refrigerator for months.

2). 10% sucrose solution was prepared to prevent bursting of pollen grains. 300 mg/l of calcium nitrate was added into the sucrose solution to improve the response of pollen systems.

3). To 2-5ml of sucrose solution in a small glass vial drops of stock solution of FDA were added until the resulting mixture shows persistent turbidity. The mixture was used within 30 min. from preparation; otherwise most of the FDA would precipitate.

4). A drop of sucrose-FDA mixture was taken on a slide.

5). Sufficient amount of fresh pollen grains were suspended in the preparation.

6). The preparation is incubated in a humidity chamber (>90%RH) for 5-10 min.

7). At the end of the incubation period, a cover glass was lowered and the preparation was observed under the fluorescence microscope with HPWB (High Performance Wide Band) filter.

8). Pollen grains that fluoresce brightly (which gave a bright, yellowish green) were scored as viable. And those that didn't fluoresce were non viable. For calculating pollen viability, total

number of viable and non-viable pollen grains was counted from 20 microscopic fields. Five replicates were maintained.

3.4 Physico-chemical Analysis

Water samples from two sites were collected and analyzed in Laboratory. "Standard Methods for Examination of Water and Waste Water" as presented by the American Public Health Association (APHA 1998) was adopted for the analysis of water samples. All the chemicals were of AR grade. Double distilled water was used for the preparation of reagents and solutions. The experimental detail of parameters considered for assessment of Physico-chemical properties of water are given below:

3.4.1 Temperature:

Temperature measurements have been made with mercury filled 110° centigrade bulb thermometer in a natural stream, the reading of which taken after 2 minutes of dipping to a depth of about 30 cms.

3.4.2 pH:

The pH value of water sample was measured by electronic method. The pH meter was calibrated with standard buffer solutions viz. potassium hydrogen phthalate (0.05 M; pH=4.01) and borax (0.01 M; pH= 9.14).

3.4.3 Total Alkalinity:

Total alkalinity of water is normally due to the presence of bicarbonate, carbonate and hydroxide salts. It is determined by volumetric method and expressed as $CaCO_3$ mg/l.

1 ml of 0.02 N H_2SO_4 = 1 mg of $CaCO_3$

Total alkalinity = (Titre value x 1000/25) mg. $CaCO_3$ /l.

3.4.4 Chloride:

Chloride content of water sample was determined volumetrically by argentometric method. From the titre value the amount of chloride ions present in the sample was calculated and expressed in terms of mg/l.

3.4.5 Total hardness:

The total hardness of water is due to the presence of soluble salts of calcium and magnesium ions. It is estimated by complexometric method using disodium salt of ethylenediamine tetra acetic acid (EDTA) as complexing method. From the end point, the total hardness of a sample could be calculated and is expressed as $CaCO_3$ mg/l.

3.4.6 Dissolved Oxygen:

The dissolved oxygen content was measured using iodometric method. The liberated iodine was titrated against sodium thiosulphate (0.025N) solution using starch as indicator. From the end point, the amount of Dissolved oxygen can be calculated.

3.4.7 Biological Oxygen Demand:

The sample was kept in an incubator at 20° for five days. The dissolved oxygen in the incubated sample has been measured as per the procedure discussed above. The Dissolved oxygen content measured under this condition is known as BOD.

3.4.8 Nitrite:

Nitrite content of water sample was determined by azoditization method. The concentration of nitrite was obtained by comparing the absorbance with the standard graph drawn with the help of series of standard nitrite solution.

3.4.9 Phosphate:

Phosphorus occurs in natural water solely as phosphates. Phosphate content was measured by stannous chloride method. It is expressed as mg/l.

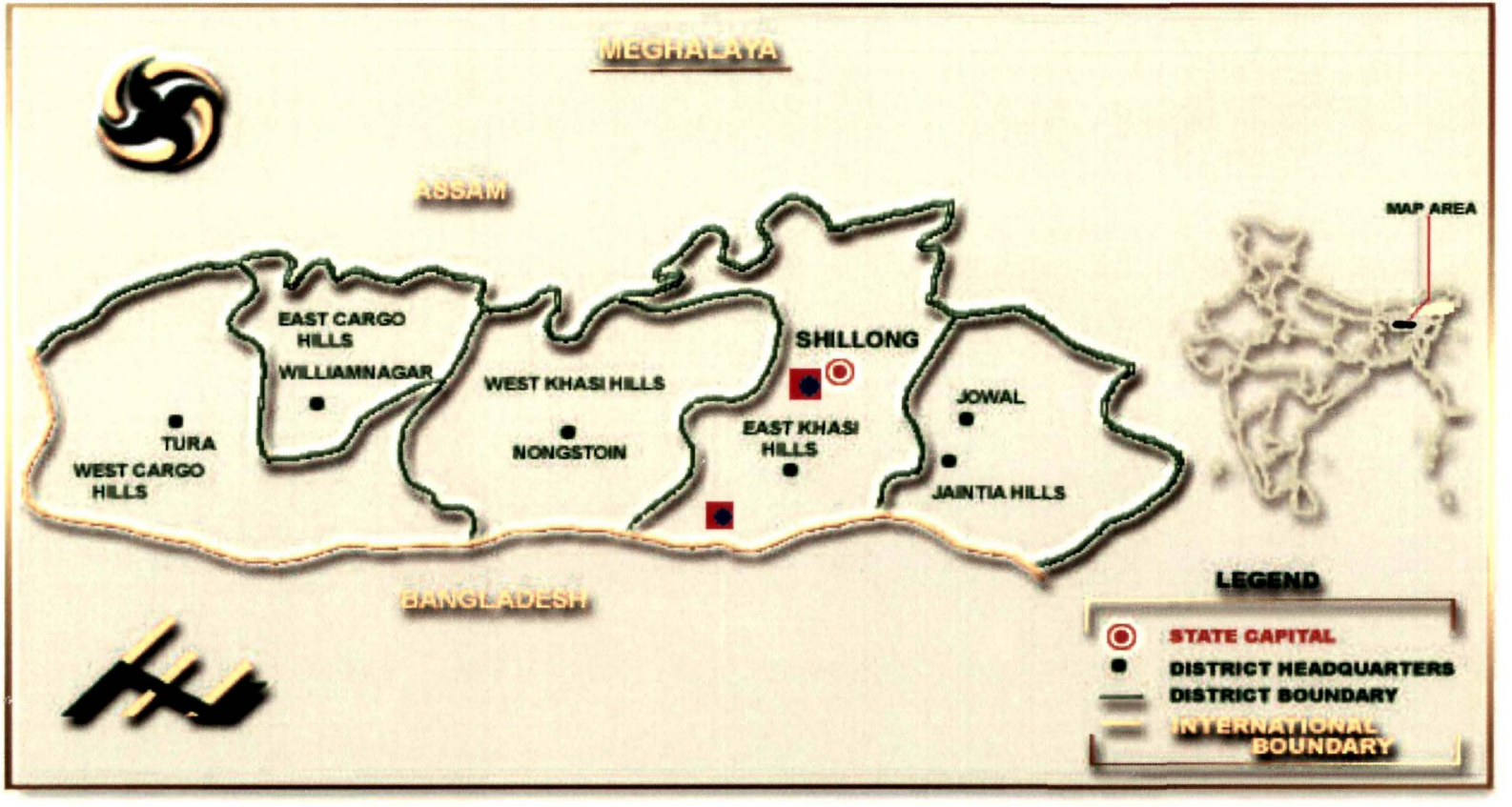
$$\text{Phosphate} = \frac{\text{mg phosphate} \times 1000}{\text{ml sample}}$$

3.4.10 Sulphate: Sulphate content of water sample was determined by turbidimetric method. The concentration of sulphate was then evaluated by comparing the optical density with the standard graph.

Plate – 3

Map showing distribution of Podostemaceae Rich Ex C. Agardh in Meghalaya

Plate 3



Distribution of Podostemaceae Rich Ex C. Agardh in Meghalaya

CHAPTER – 4

Hydrobryum griffithii

Hydrobryum griffithii (Wallich ex Griffith) Tulsane in Ann. Sc. Nat. Ser 3, xi. 104, and Monogr. Podost. 141; Wedd. in DC. Prodr. xvii. 67. *Podostemon Griffithii*, Wall. Mss.; Griff. In As. Res. xix. 105, t. 17, and Ic. Pl. As. T. 541, f. 2, & t. 544. P. *Griffithii*, Gardn. in Calc. Journ. Nat. Hist. 1850, 40, 41.

4.1 Morphology and Habitat

4.1.1 Habit

The plants are aquatics and seasonal; growing on rocky surfaces in tropical streams, in rapid, but not deep water (Plate 4.1a& b); more or less ovoid to circular outline. Small, coriaceous dark green, frond-like lobed patches, about 2.5 cm; closely attached to substrate (Plate 4.1d). Spreading over stones, sending up buds clothed at the base with distichous scale-like leaves, imbricate, enlarged at the base, tips caducous (Plate- 4.1e). Vegetative shoots very short; leaves linear, green to brownish up to 12 mm long; scattered in groups of 2-8 on the upper surface of the thallus (Plate 4.1c). Flowering takes place aerially when the water level recedes at the end of monsoon (Plate 4.1h)

Spathella boat-shaped, splitting irregularly on the upper side and at the tip. Spathe encloses the young flower, usually 3-4 mm long and 2-3 mm broad. Flowers subsessile, remaining within the bracts (Plate- 4.1f); zygomorphic, pedicellate, pedicels 2-6 mm long . Stamens 2, borne on an andropodium; as long as the ovary. Anthers oblong, golden yellowish, 1-1.5 mm long and 0.4-0.6 mm broad (Plate- 4.1j). Staminodes 2, 3.8- 4.5 mm long, transparent with subspathullate tips, appressed to the ovary in bud (Plate- 4.1i). Ovary ob-triangular, 4.5-6.0 mm long and 1.5-2.0 mm broad, green (Plate 4.1g). Stigmas bifid, wedge-shaped, brownish, unequal, granular, subulate, 1.0-1.5 mm long and 0.2-0. 5 mm broad (Plate- 4.1 j).

Fruit a capsule, isolobous, 12-ribbed, 3-5 mm long; 2-locular, opening by two equal valves (Plate- 4.1j& l). Stigmas 2, entire (Plate- 4.1g). Seeds minute, numerous; elliptical-patelliform, the surface granular, testa membranous and transparent, tegmen brownish; size ranges from 280-315μ long, and 160-190μ broad, numerous (Plate- 4.1m & n). The outer integument of seed, when get wet, becomes mucilaginous and stick to the rocks. Seed attachment and seedling establishment takes place under the current of water (Plate- 4.1b). Flowering starts in late September and fruiting observed in late November.

4.1. 2 *Locality*

Specimen collection is carried out in stream at fossil park, Janiaw, in Lawbah region (Plate- 4.1a); about 6 km away from Mawsynram, located in East Khasi Hills District, Meghalaya State, India (91° 70'N – 25° 40'E).

The species has been reported in Indo china, N. Vietnam, Bhutan, Myanmar, Nepal and India; Assam, Manipur, Nagaland, Sikkim, Uttar Pradesh and West Bengal from India.

4.1.3 *Key characters for Identification*

1. Cotyledons two.

   -Dicotyledons

2. Fruit a capsule. Plants attached to rocks by disc like processes; appearing like algae or bryophytes.

   -Podostemaceae

3. Perianth of 2, rarely 3 scales. Stamen 1 or 2-3 with a filament connate.

   -Eupodostemaceae

4. Thallus foliose (lichen-like), closely attached to substrate; flowers subsessile, remaining within the leaves; spathella boat- shaped.

- Hydrobryum

5. Leaves of floral shoots scale-like, distichous, imbricate, and enlarged at the base, tips cadocous. Fruit capsule, isolobous, 12-ribbed.

H. griffithii.

Plate 4.1

a Habitat in Lawbah. The plants are growing in the cataracts. Note the dry thallus of last year's growth on the sides.

b Seed attachment and seedling establishment under the current of water. The outer mucilaginous integument is responsible for attachment to the rocks.

c A part of vegetative plant with filiform leaves (L).

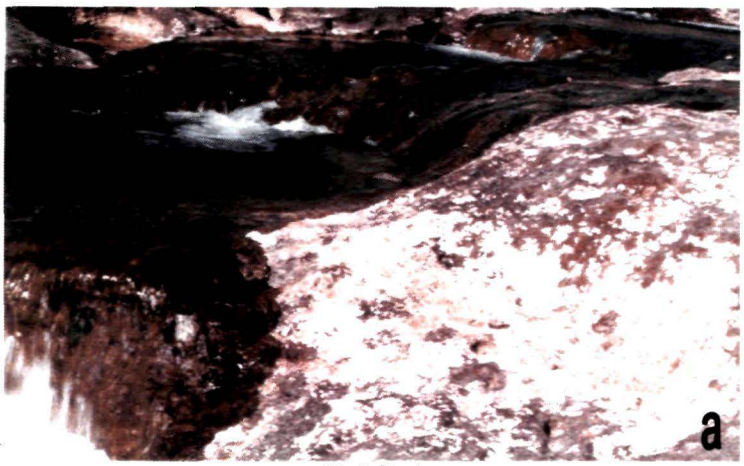
d Crustose thalus with young flower buds.

(Magnification- b= x 25; c& d= x ½)

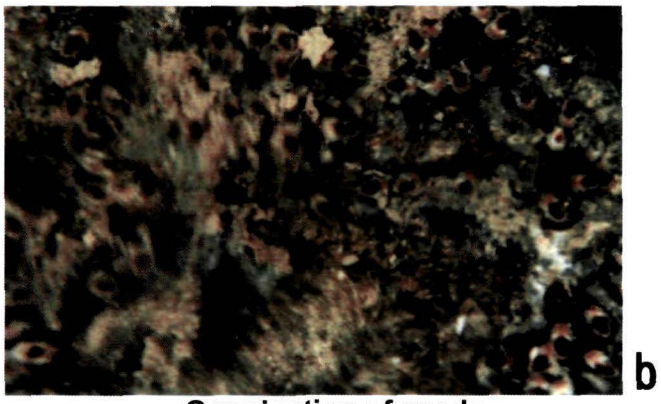
Plate- 4.1

***Hydrobryum griffithii* (Wall. ex Griff.) Tul.**

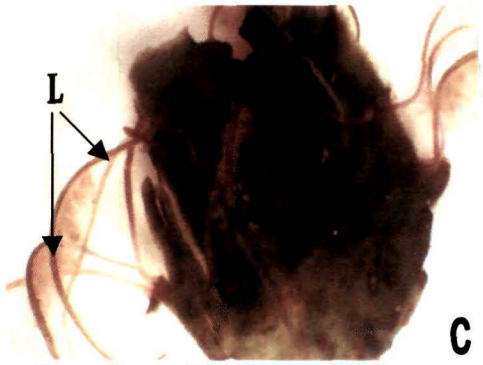
Morphology and Habitat



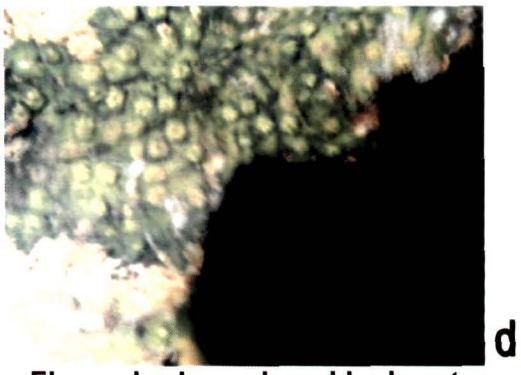
Habitat



Germination of seeds



A portion of thallus with leaves



Flower buds enclosed by bracts

Plate- 4.1

e An enlarged view of thallus with young flower buds.

f S E M photograph of young flower . The bud (O) is enclosed by 5-7 bracts (Br).

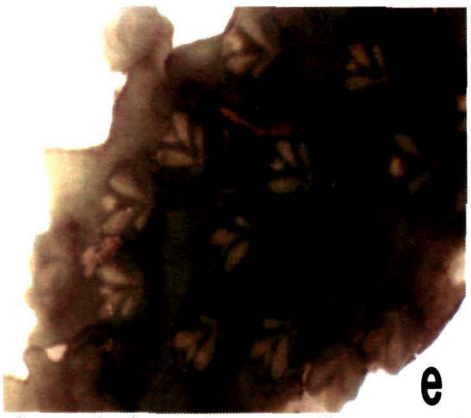
g Bisexual monoecoius flower in the natural habitat. Note the biforked filament (F), borne at the base of ovary (O and the bifid sigma (Sm).

h Flowers in bloom. Note the distinct ovaries and stamens in the thallus.

(Magnification- e= x 9; g= x 9; h= x 2)

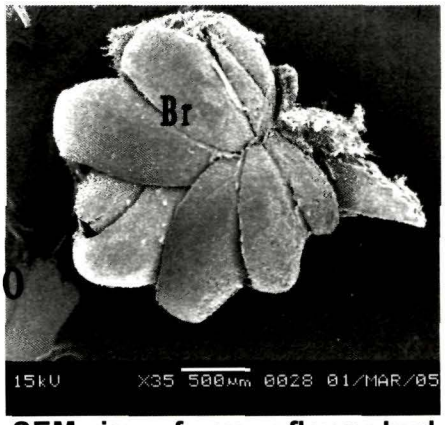
Plate- 4.1

Hydrobryum griffithii (Wall. ex Griff.) Tul.



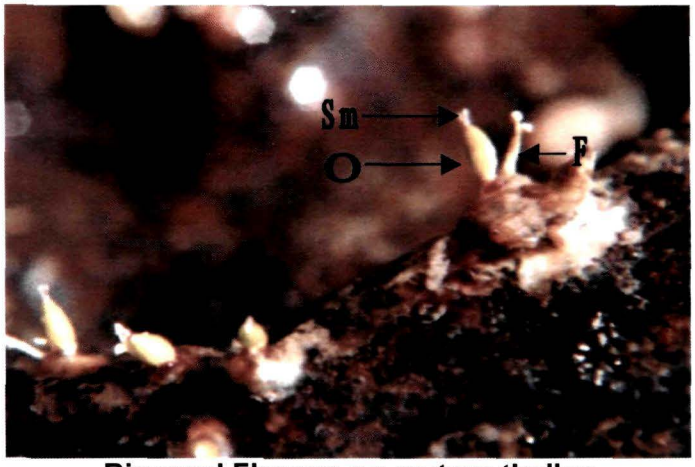
e

Enlarged view of young flower buds



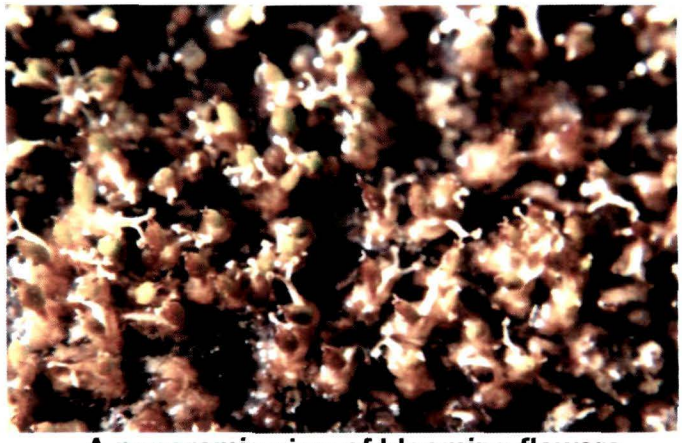
f

SEM view of young flower bud



g

Bisexual Flowers on mature thallus



h

A panoramic view of blooming flowers

Plate- 4.1

i& j Bisexual flower in SEM and light microscopy. The filament (F) is appressed to ovary (O) when young and get spread at maturity. See the brown colored bifid stigma (Sm). Note the stamen (S) and staminodes (St) borne on either side of the filament.

k& l Dry thallus with capsules on barren rocks at the end of the monsoon period.

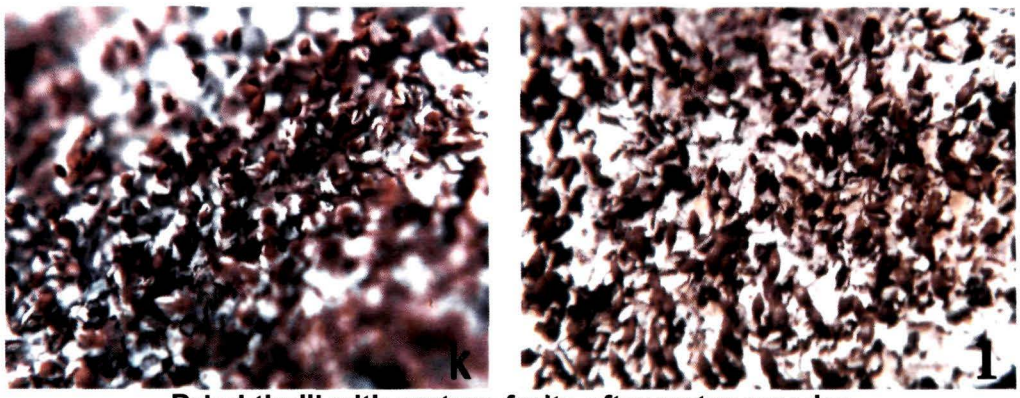
m& n SEM photograph of seeds. The seeds are more 0r less rectangular in shape with ridges and furrows.

(Magnification- i& j= x 26: k& l= x 2)

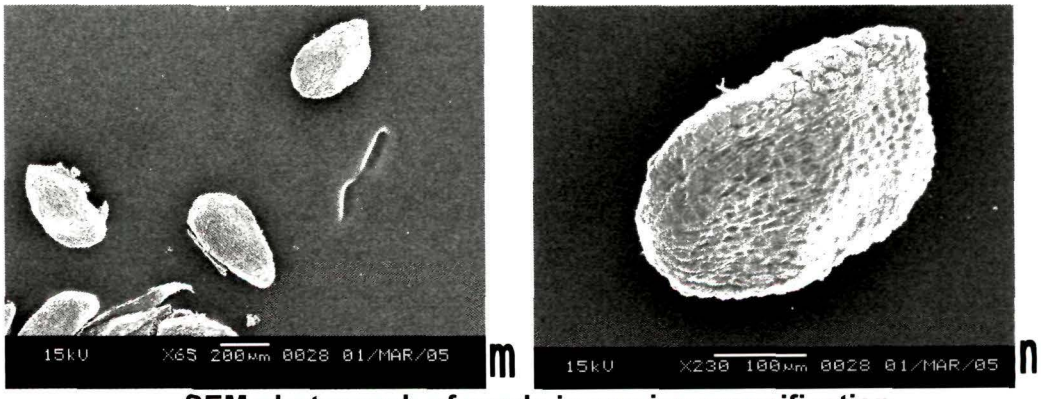
Plate- 4.1

Hydrobryum griffithii (Wall. ex Griff.) Tul.

Enlarged view of flower under SEM and light microscopes



Dried thalli with mature fruits after water recedes



SEM photograph of seeds in varying magnification

4.2 Anther Development and microsporogenesis

4.2.1 *Initiation of Microsporangium*

The two anther primordia initiate far before the development of gynoecium and enclosed by a distinct membranous perianth lobe (Plate 4.2 a). The anther primordium comprises a mass of undifferentiated meristematic cells covered with a unilayered dermatogen, thus conforming to tunica-corpus concept. The two anther primordia grow in height by intercalary meristematic activity of andropodium to form biforked filament (Plate 4.2 c). After attaining a height of 10- 13 µm, the four anther primordial loci are differentiated on the adaxial side, so that all the four lobes of each anther are facing towards the stigma (Plate- 4.2 d). The flowers of *Hydrobryum griffithii* are bisexual which have two tetrasporangiate anthers.

4.2.2 *Archesporial cell and Anther wall formation*

A row of 3-4 cells in the hypodermis differentiated into an archesporium in each anther lobe. Each archesporial cell is very conspicuous with dense cytoplasm and a large prominent nucleus (Plate- 4.2b). The already differentiated archesporial cells divides anticlinally to produce more archesporial initials. Each archesporial initial divides periclinally producing two cells of unequal size. The much larger inner row of cells became primary

sporogenous cells and the smaller outer cells form primary paritel cells (Plate 4.2 e).

The primary parietal cells undergo only one periclinal division to form two-layered secondary parietal layer. The outer one functions as endothecium, while the inner one directly transformed into tapetum (Plate- 4.2 e). Middle layers are absent. The endothecial layers acquire thickenings only when the pollen grains are formed. More or less all the cells of anther lobe bear thickenings except the vasculature. The cells of endothecium and tapetum are fully laddened with starch grains. Callose deposition in the meiocytes separates the individual microspore mother cells, enclosed within the callose wall layer (Plate- 4.2 f & g).

4.2.3 *Tapetum*

The entire tapetum is derived from the inner layer of secondary parietal layers and is responsible for the nourishment of developing microspores. Tapetum is of secretory type, uninucleate as well as binucleate (Plate 4.2 f& g); and persists till the formation of pollen grains in pairs (Plate 4.2 k). The cells are densely cytoplasmic with starch and protein contents. The nuclei of tapetum become comperatively larger in size.

4.2.4 *Meiosis and Cytokinesis*

The pollen grains are arranged in isobilateral and decussate arrangement. Meiosis is uniform in all the microspore mother cells within the thecum, but not in adjacent anther locule (Plate 4.2 h). The callose wall gets dissolved with the completion of cytokinesis, but before the start of pollen wall deposition. During the onset of meiosis in the microspore mother cells, nuclear spindle appears on either side of metaphase nucleus; as a result, a binucleate microspore formed (Plate 4.2 i). Simultaneously, centrifugal cell plate formation is initiated in a transverse plane that a dyad is resulted (Plate 4.2 j). While microspore mother cells are undergoing meiosis, the adjacent tapetal cells show granular appearances, which indicate the synthesis of orbicules or pro-ubisch bodies (Plate 4.2 k).

The nuclei of dyad undergo Meiosis-II; with which, the tapetum becomes binucleate (Plate 4.2 k). The extent and progress of cell division was not uniform in the two sister nuclei. The microspore dyad then becomes four nucleate and simultaneously a cell plate is formed in between the newly formed microsporocytes. So that a tetrad is formed arranged either in opposite decussate (Plate 4.2 l) or isobilateral configuration (Plate 4.2 m). The nucleus enlarges and lies in the centre, but the cytoplasmic contents do not increase rapidly. The tapetal cells started disorganizing with the formation of tetrad after meiosis- II.

The tetrad then separated either in a horizontal plane or vertical plane to produce a pair of pollen grains. Callose wall around the tetrad remain for quite sometime (Plate 4.2 m) and disappear when the pollen grains are shed in pairs (Plate 4.2 n).

Subsequently, the cytoplasmic content increases rapidly and the nucleus moves to the distal end, thereby forming vegetative and generative cells. The shape of a pollen grain is established with thick rigid pollen wall and shows a tapering end in one side of the double pollen grain (Plate 4.2 n). Because of the callose wall deposition, the pollen grains give fluorescence with aniline blue under fluorescence microscope (Plate 4.2o). Germinated pollens are observed on the stigmatic surface in the natural conditions (Plate- 4.2 q).

4.2.5 *Pollen grains*

Pollen grains of *Hydrobryum* are dyads (Plate 4.2n). The dyad is about 20- 25µm long and 9-11 µm broad. Individual grains of dyad are subprolate in structure and triaperturate with apertures aligned between grains of the dyad. Pollen grains are circular in polar view The apertures often called colpi have weakly defined margins and range in morphology from oblong furrows with broadly rounded ends to large oval shaped pores. Ornamentation microechinate with processes of different size including pads.

4.2.6 *Pollen viability*

The viability of pollen was studied with Flourochromatic Reaction (FCR) test and is expressed in terms of percentage. The viable pollens fluoresce brightly and non-viable one didn't fluoresce (Plate 4.2 p). Reading was taken in such a way that ten microscopic fields were observed in H. *griffthii*, the mean value of which was taken as a total number of pollen grains. *Hydrobryum griffithii* shows moderately high pollen viability. The test revealed that 73.68 % of pollen grains were viable.

Table – 4.2.6: Pollen viability test by using FCR Test

Name of Plant	Total no. of pollens		No. of viable pollens		Percentage of viability
Hydrobryum griffithii (Wallich ex Griffith) Tul	R_1	98	R_1	72	73.68 ± 7.12
	R_2	73	R_2	52	
	R_3	110	R_3	81	
	R_4	84	R_4	58	
	R_5	167	R_5	129	
	Mean	106.4	**Mean**	78.4	

± : Standard deviation (SD)

R_X: Number of replicates

Plate- 4.2

a Longitudinal section (LS) of young flower showing two staminal primordia (Sp) arises as an independent unit, enclosed within a perianth (Pr).

b Transverse section (TS) of young anther lobe with a plate of hypodermal archesporial initials (Ai).

c LS of young flower bud showing intercalary meristematic growth in the andropodium (Ap), the filament (F) becomes bifid.

d L. S of young flower bud showing gynoecium (G) and anther lobes (An) with sporogenous cells, which appears darkly stained inside the anther locule.

e TS of anther lobe showing a mass of sporogenous cells (Sc), enclosed within wall layers- tapetum (Tp), endothecium (Em) and epidermis (E). Middle layer is absent.

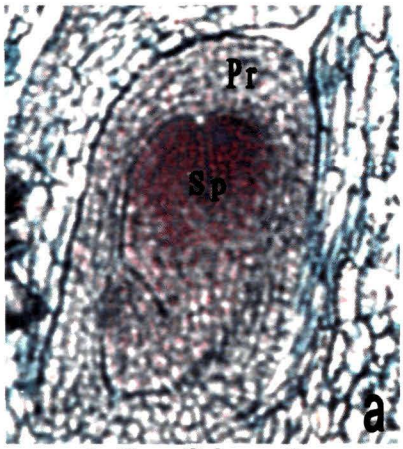
f& g TS of anther lobe. on the microspore mother cells sepearte nucleusCallose deposition separates the individual cells (mmc). Note the uni- and binucleate tapetum (Tp).

(Magnification- a= x 100; b= x 180; c= x 55; d= x 50; e= x 185; f= x 320; g= x 530)

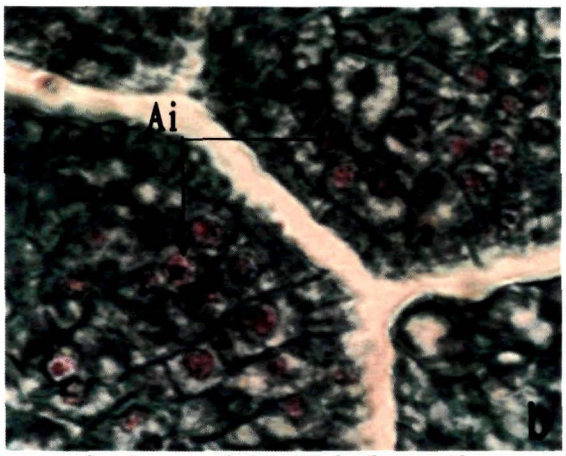
Plate- 4.2

***Hydrobryum griffithii* (Wall. ex Griff.) Tul.**

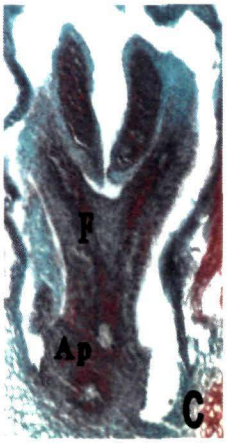
Anther Development and Microsporogenesis

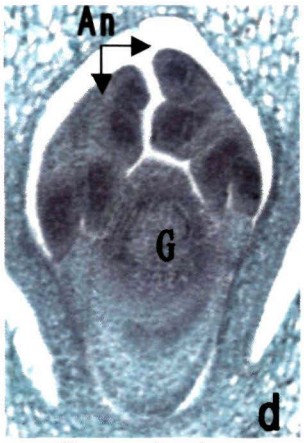


Anther Primordia

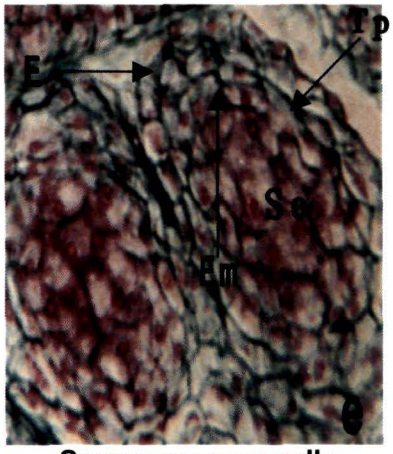


Archesporial initials formation

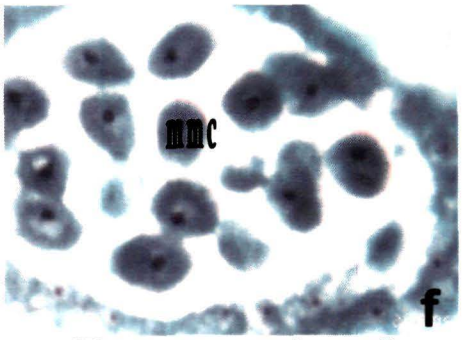




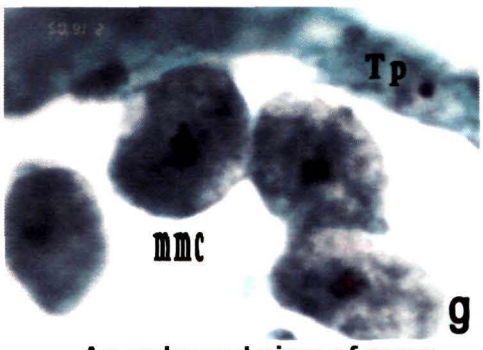
L.S. of young flower bud



Sporogeneous cells



Microspore mother cells



An enlarged view of mmc

Plate- 4.2

h& i TS of anther lobe exhibits metaphase figure of Meiosis-I in microspore mother cells. Note the tapetum (Tp); And anaphase stage of microspore mother cell during meiosis -I.

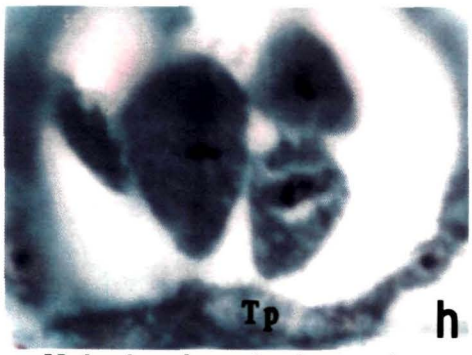
j& k Formation of Cell plate after Meiosis- I. The dyad stages (D) within a binucleate tapetum (Tp).

l& m Meiosis-II results in tetrad formation. Tetrads arranged in opposite decussate and isobilateral configurations.

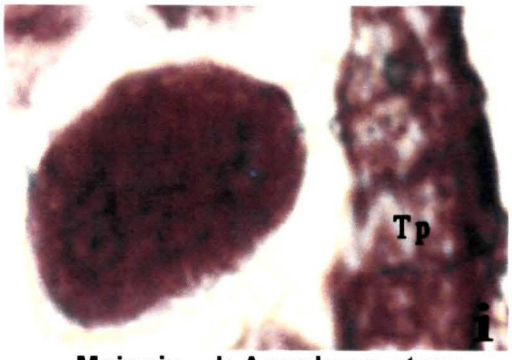
(Magnification- h= x 500; i= x 800; j& k= x 800; l& m= x 550)

Plate- 4.2

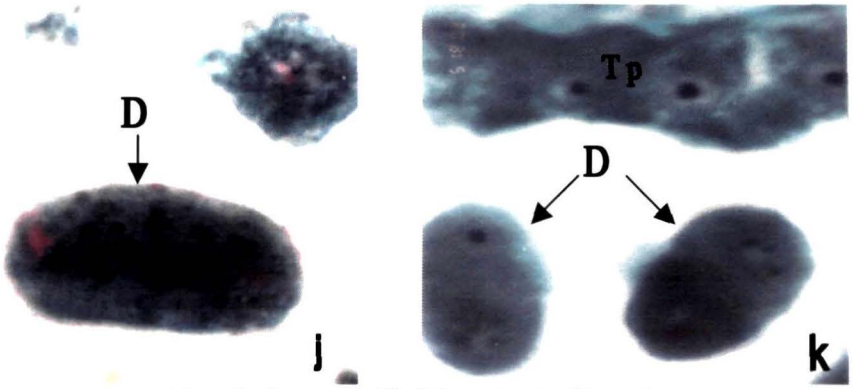
***Hydrobryum griffithii* (Wall. ex Griff.) Tul.**



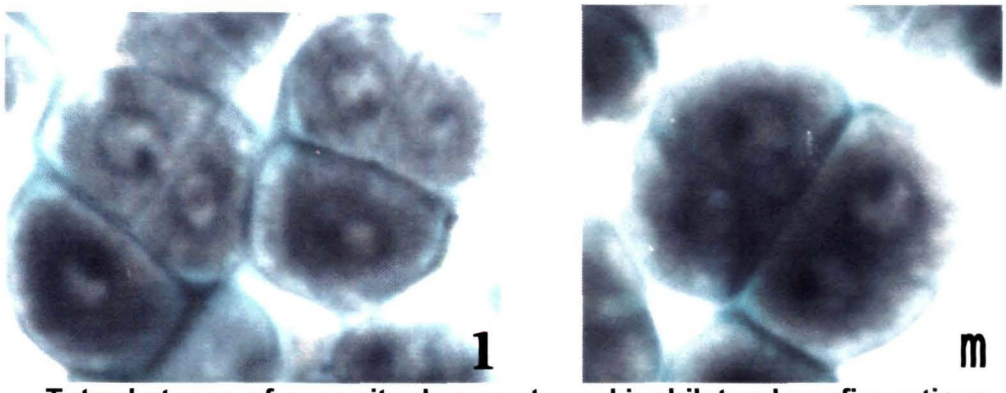
Meiosis – I; metaphase stage



Meiosis – I; Anaphase stage



Dyad stages with binucleate Tapetum



Tetrad stages of opposite decussate and isobilateral configurations after Meiosis - II

Plate- 4.2

n A single dyad pollen grain. Each grain is bicellular.

o The pollen grains emits fluorescence in aniline blue because of callose deposition.

p Pollen viability test in FCR Test. Only the viable ones emit florescence with Flourescin di-acetate (FDA).

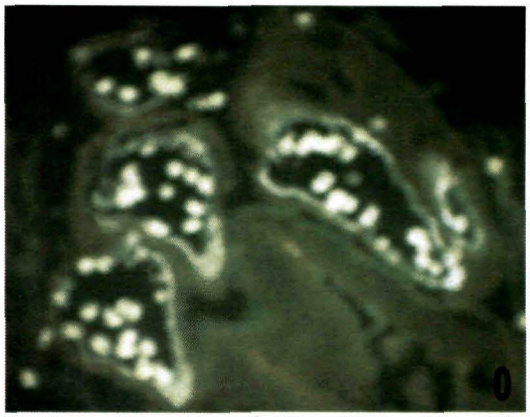
q Squash preparation of stigmatic lobes showing pollens germinated on the stigma (Sm) in natural condition.

(Magnification- n= x 1500; o= x 100; p= x 200; q= x 325)

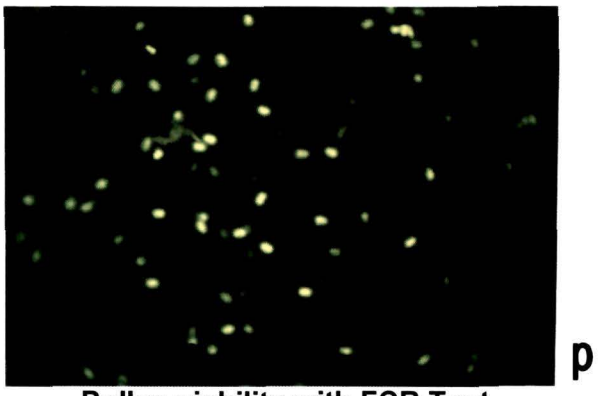
Plate- 4.2

Hydrobryum griffithii (Wall. ex Griff.) Tul.

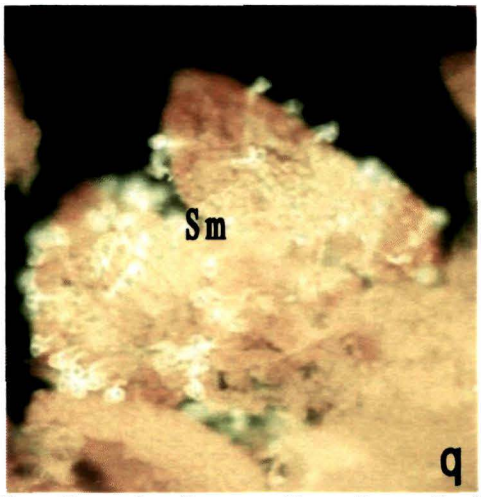
Pollen grains in pair



Fluorescence of callose with aniline blue



Pollen viability with FCR Test



Pollen Germination on the stigmatic lobes

4.3 Megasporogenesis and Embryo sac deveopment

4.3.1 *Megasporangium*

Ovules numerous, anatropous, tenuinucellate, bitegmic on axile placentation (Plate 4.3a & b). The ovules arise on the placenta as a tiny protuberance, after reaching 50 µm in height (Plate 4.3 c). The abaxial side of the cells divides much faster than the opposite side. As a result, the ovule primordium bends at an angle of 90° (Plate 4.3 d). Further curvature takes place due to the differentiation of chalazal and nucellar tissues. The chalazal portion plays major role for the incorporation of cells into the vertically oriented unilayered nucellar tissue as well as the differentiation of inner integument (Plate 4.3 e).

At this point of time, the outer integument is initiated due to rapid growth in the chalaza portion; the ovule grows and undergoes a curvature of 180° and forms the micropyle. The accumulation of starch grains in the outer and nucellar tissues is prominent soon after the bitegmic condition is attained (Plate 4.3 e).

A hypodermal cell is differentiated from the nucellar apex as an archesporial initial. The archesporial initial is densely cytoplasmic with distinct nucleus and nucleolus (Plate 4.3 f). Soon it gets rounded off and separated with the nucellar epidermis from all sides. As soon as the archesporial cell is differentiated, the

inner integuments start to develop from the chalaza. Due to the differentiation and further growth of inner integument, the archesporial initial moves towards the micropylar region (Plate 4.3 g). So that, the archesporial cells lie in between the outer and inner integuments.

4.3.2 *Megasporogenesis*

The archesporial cell directly functions as megaspore mother cell; nuclear spindles appear in the equatorial plate (Plate 4.3 h). It undergoes meiosis-I to form dyad cell (Plate 4.3 i). The micropylar cell of the dyad disintegrates and persists as a cap on the micropylar end until the development of early embryogenesis. The subsequent division of meiosis-II is homeotypic division, followed by cell plate formation (Plate 4.3 j). Out of these two cells, chalazal one is smaller than the micropylar one just below the disintegrated dyad cell (Plate 4.3 k). The chalazal nucleus of dyad slowly degenerates thereby forming a single functional micropylar megaspore (Plate 4.3 l). This uninucleate megaspore is the starting point for the further development of female gametophyte. Therefore the female gametophyte development in *Hydrobryum* belongs to Allium type.

Concomitant with female gametophyte development, several changes occurs in the inner integument- 1)- It enlarges laterally to form the cavity in the middle of the ovule, so as to accommodate

the nucellar plasmodium. 2) - The inner layer of inner integument acquires wall ingrowths and act as transfer cells and functions as endothelium as well. The further development of embryo sac originates from the remaining single cell. Therefore, the female gametophyte development in H. *Griffithii* is monosporic type. The functional megaspore undergoes two cycles of mitosis to form the four nuclei that constitute the embryo sac (Plate 4.3 m).

4.3.3 *Embryo sac development (Megagametogenesis)*

The centrally located functional megaspore i.e. in between the two degenerated cells, divide twice without wall formation, as a result of which a four nucleate condition is formed (Plate 4.3 m). Therefore, altogether 4 nuclei are formed which take part in the organization of embryo sac. The extreme chalazal one functions as polar nucleus, the two micropylar nuclei constitute synergid and the remaining nucleus in the middle function as egg cell (Plate 4.4 a). The egg cell is the largest cell in the embryo sac. The development and differentiation of embryo sac in H. *Griffithii* is 5 nucleate, 4 celled structure (Plate 4.4 b) of Apinagia type B.

Plate- 4.3

a- c L.S of flower showing stigma (Sm), gynoecium (G), placenta (Pl) and anatropous ovules (O) with an outer integument (Oi).

d Ovule primordium with a tenuinucellate curvature of funiculus.

e Hypodermal cell differentiates to form archesporial initial (Ai). Note the tenuinucellate condition (Tn) of the ovule.

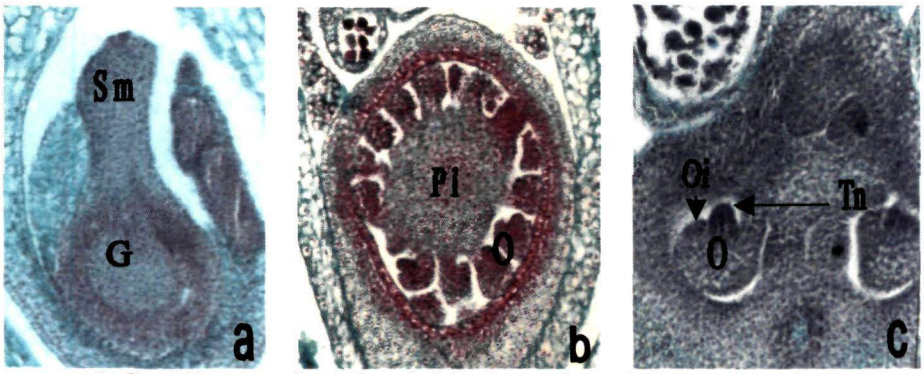
f& g Archesporial cell directly function as megaspore mother cell (MMC). Note nucellus (Nu) and the initiation of inner integument (Ii). Also note the separation of MMC from the nucellar epidermis.

(Magnification- a= x 120; b= x 240; c= x 600; d= x 750; e= x 730; f= x 550; g= x 800)

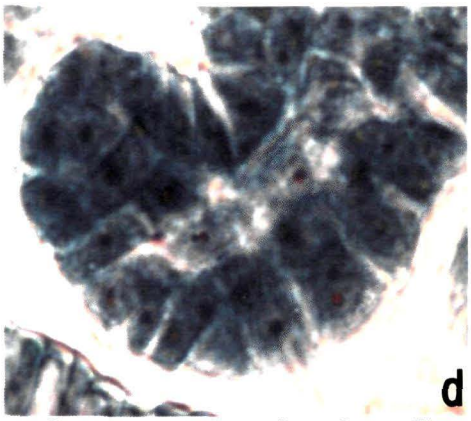
Plate- 4.3

Hydrobryum griffithii (Wall. ex Griff.) Tul.

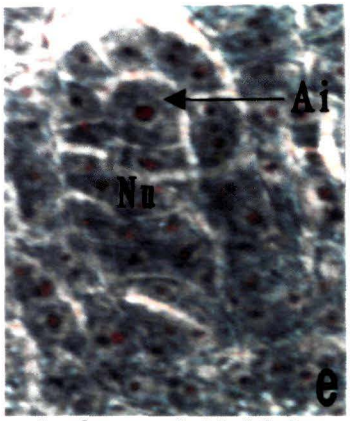
Megasporogesis and Embryo sac formation



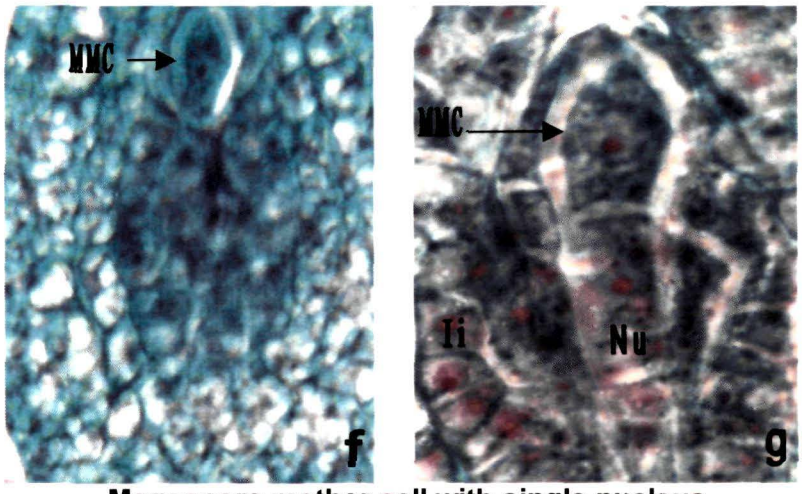
L.S of flower showing initiation of anatropous ovules



Curvature of ovule primordia



Archesporial initial



Megaspore mother cell with single nucleus

Plate- 4.3

h& i Meiosis – I in megaspore mother cell with nuclear spindle (Ns) so as to form a two-nucleate dyad (D) as seen in 'i'.

j Meiosis – II to form a chalazal dyad (Cd) with degerated dyad (Dd) at the micropylar end appear pink in colour. The darkly stained region is the mucilaginous mass.

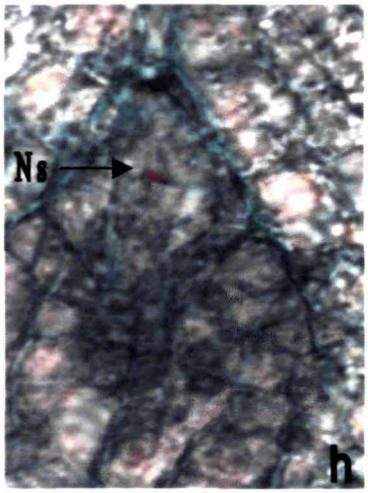
k& l The chalazal nucleus (Dn) degenerates. The micropylar nucleus forms the functional (Mr) megaspore. See the degenerated dyad (Dd) at the micropylar end.

m Four nucleate embryosac. Egg apparatusl (Eg) comprises of two synergids and egg cell. Polar nucleus (Pn) occupies chalazal pole.

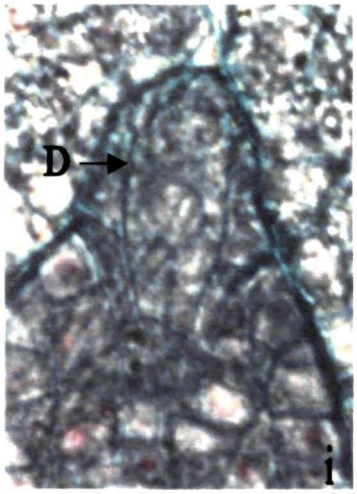
(Magnification- h& i= 1350; j= x 1350; k= x 1350; l= x 1800; m= x 1800)

Plate- 4.3

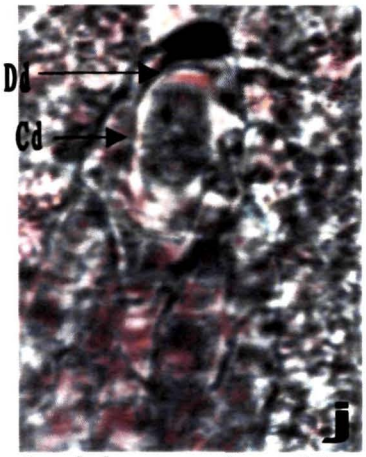
***Hydrobryum griffithii* (Wall. ex Griff.) Tul.**

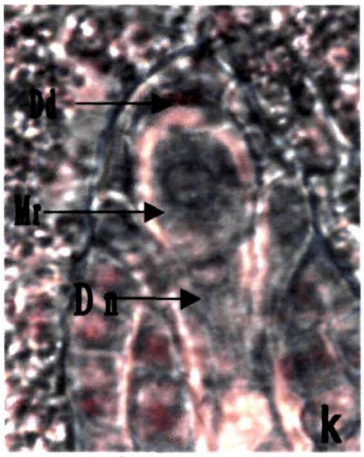


Division of MMC

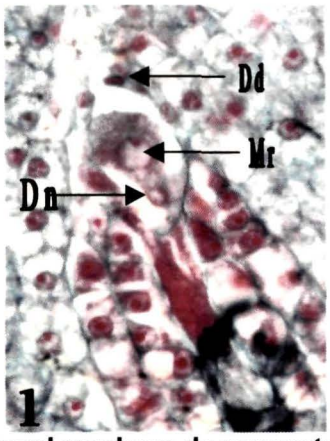


Two nucleate condition of MMC

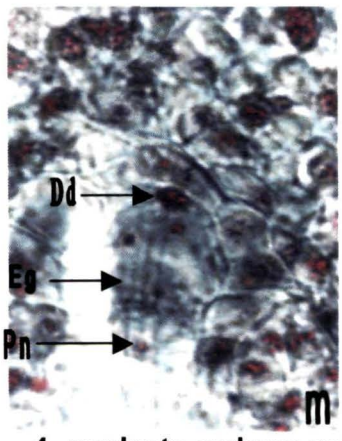




Upper dyad degenerate and lower one undergoes one more division



Chalazal nucleus degenerated



4- nucleate embryo sac

4.4 Embryogeny

4.4.1 *Zygote*

After fertilization, the entire embryo sac is occupied by the zygote (Plate 4.4e); because of its increasing volume, thereby pushed the polar nucleus toward the chalazal end; which, on the verge of disintegration and still can be noticed towards chalazal end (Plate 4.4 f). The zygote shows distinct polarity in the distribution of nucleus and sub-cellular organelles towards the chalazal end. While reserve storage products such as PAS-positive particles are at the micropylar end (Plate 4.4 c). The disintegrated synergids and the dyad cell persist as dark structures (Plate 4.4d). The entire protoplast of the zygote is enclosed by a thin cellulosic cell wall (Plate 4.4 e).

4.4.2 *Embryogenesis*

The zygote divides in transverse plane to form two celled proembryo i,e. the apical (ca) and basal cell (cb), the former is smaller than the latter (Plate 4.4 h). However, the apical cell (ca) stains deeply with distinct nucleus and has dense cytoplasm, but the basal cell (cb) takes considerably less stain.

The 'ca' is located towards the chalazal pole and the 'cb' is located towards the micropylar end. The basal cell 'cb' undergoes division in a transverse plane to form two superposed cells of 'm'

and 'ci'. Almost, simultaneously the 'ca' undergoes vertical division, so that the resultant daughter cells of 'ca' are juxtaposed in the same tier. Thus the tetrad has T-shaped configuration (Plate 4.4 j& k.). The tetrad stage is followed by another vertical division in a plane at right angle to the first vertical division in the distal cell 'ca'. These four juxtaposed cells constitute the quadrant 'q', which again undergoes transverse division so that an octant is formed. The cells of the octant is arranged in two tiers, I and I' (Plate 4.4 l).

The further division of cells in the octant at various planes led to the formation of globular embryo (Plate 4.4 m). At this stage, cotyledon loci 'Pco' are initiated which grow to form heart shaped and later on torpedo shaped embryo. The derivatives of 'cb' such as 'm' and 'ci' are stained faintly (Plate 4.4 j). The cell 'm' undergoes vertical division to form two juxtaposed cells above the quadrant or octant (Plate 4.4 l). While 'ci' divides only once or rarely twice to form a linear row of 2 or 4-celled suspensor. The distal cell of the suspensor is transformed into haustoria (Plate 4.4 o). Therefore, the embryogeny of H. *griffithii* followed the Onagrad type of ontogeny.

4.4.3 *Suspensor Haustorium*

The most distally placed cell n' divides vertically to form two juxtaposed celled structure. These two cells produce several

haustorial branches that penetrate the micropyle as well as in between the integuments and the haustorial branches reach up to the chalazal portion (Plate- 4.4 o). The nuclei of two suspensor cells are hypertrophied with distinct nucleolus (Plate- 4.4 l).

4.4.4 *Nucellar Plasmodium*

At the time of embryo sac differentiation, the cell wall of nucellar tissue start to disappear and the entire protoplast fuses to form the coenocytic plasmodium (Plate 4.4 i). Simultaneously the outer and inner integument started expanding laterally to form the ovoid cavity in the middle of the ovule so as to accommodate the nucellar plasmodium. The entire mass of nucellar plasmodium is enclosed within the cuticular sac of endothelial origin (Plate 4.4 n).

4.4.5 *Endothelium*

The inner layer of inner integument differentiates into endothelium as soon as fertilization is over in H. *griffithii.* At the time of differentiation of endothelium, the cells elongate radially and the cell walls become thick, the cytoplasm is richly dense with prominent bi-nucleate condition (Plate 4.4 i). The food reserves such as starch grains and proteins are stored in the endothelium in the later stage; the cytoplasm becomes thin and the nuclei exist as such, but masked by lot of food reserves. The endothelium is confined to only three fourth of the ovule excluding a few cells at the micropylar as well as the chalazal portion. The endothelium is

separated from nucellar plasmodium and embryo sac by a thin layer of cuticle (Plate- 4.4 g). As the embryo develops, the cuticle of endothelium recedes and separated from the endothelium to form a sac like structure within which only the nucellar plasmodium is present (Plate 4.4n).

The nucellar plasmodium is short-lived, up to the globular stage of embryo development. The further development of embryo is dependant upon the nutrition supplied by the endothelium as well as suspensor haustoria. Endothelium exists up to the differentiation of complete embryo

Plate- 4.4

a& b Mature embryosac in different magnifications. Egg apparatus (Ea) comprises two micropylar synergids (**Ss**) and a central egg cell (Ec) with polar nucleus (Pn). Note the presence of fully differentiated endothelium (En) distinct in 'a'. (x 300 and 2500).

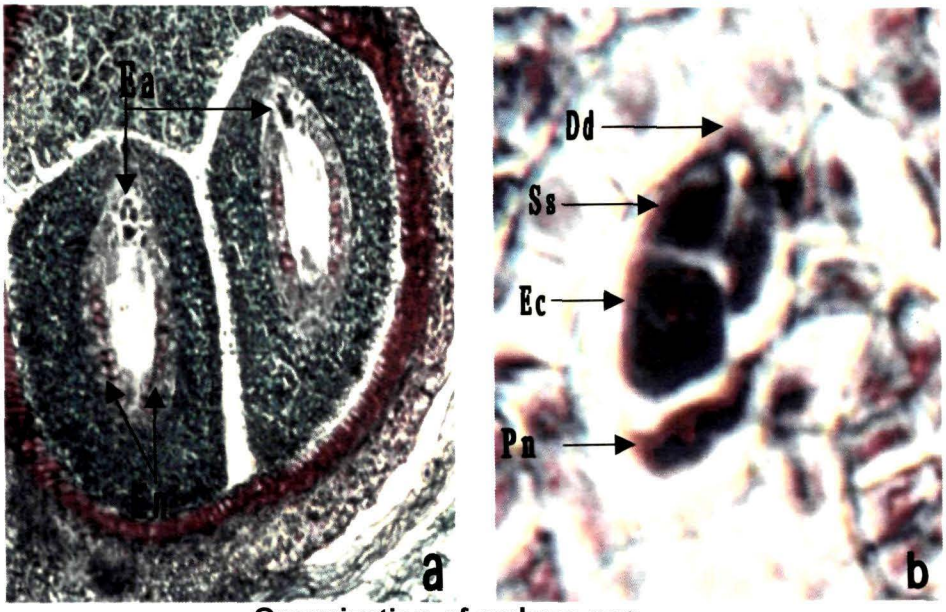
c& d Fertilized egg or zygote (Zy) with distinct nucleus and nucleolus, degenerated dyad (Dd), degenerated synergid (Ds) and degenerated polar nucleus (Dpn). Note the distinct polarity of the zygote. (x 1800)

(Magnification- a= x 300; b= x 2500; c& d= x 1800)

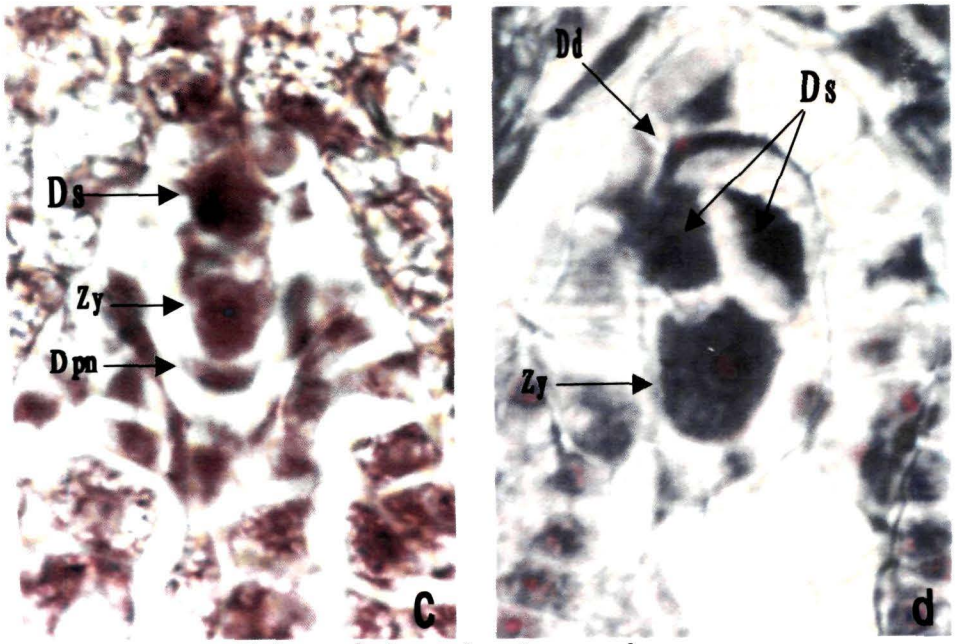
Plate- 4.4

Hydrobryum griffithii (Wall. ex Griff.) Tul.

Embryogeny



Organization of embryo sac



Enlarged view of mature embryo sac

Plate- 4.4

Median longitudinal section of ovule showing;

e Zygote (Zy) occupying the entire embryo sac. Note the starch grains and most cytoplasm concentrated near chalazal pole. Note the degenerated synergid (Ds) and degenerated dyad (Dd). (x 2000)

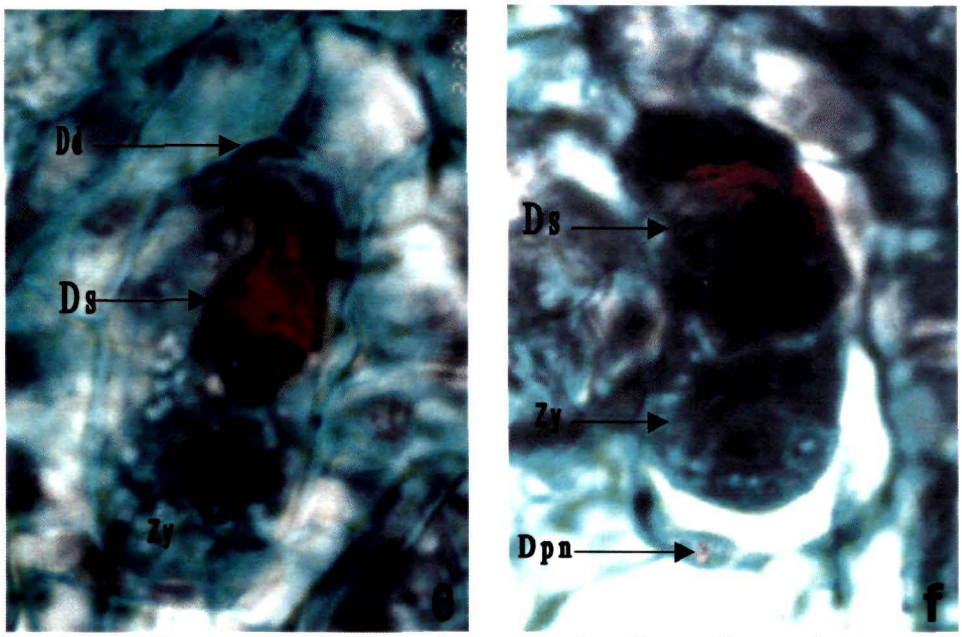
f Another view of Zygote (Zy) with degenerated synergids (Ds), degenerated dyad cell (Dd) and degenerated polar nucleus (Dpn).

g SEM photograph of TS of ovule showing nucellar plasmodial region (Np) enclosed within endothelium (En).

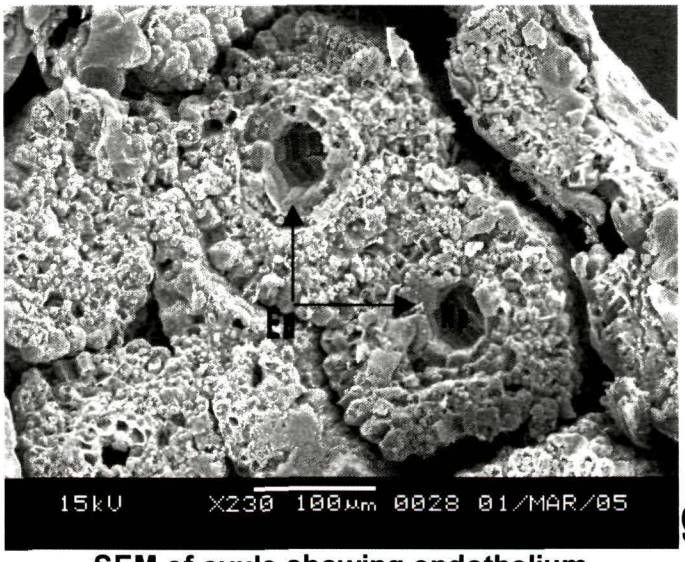
(Magnification- e= x 2000; f= x 2000)

Plate- 4.4

Hydrobryum griffithii (Wall. ex Griff.) Tul.



L.S of flower showing zygote occupying the entire embryo sac.



g

SEM of ovule showing endothelium

Plate- 4.4

Median longitudinal section of ovule showing:

h& i Two celled-proembryo with apical cell (ca) and basal cell (cb). The dark regions represent degenerated dyad (Dd) and synergids (Ds) in the micropylar end. Note the presence of Nucellar plasmodium (Np) and Endothelium (En).

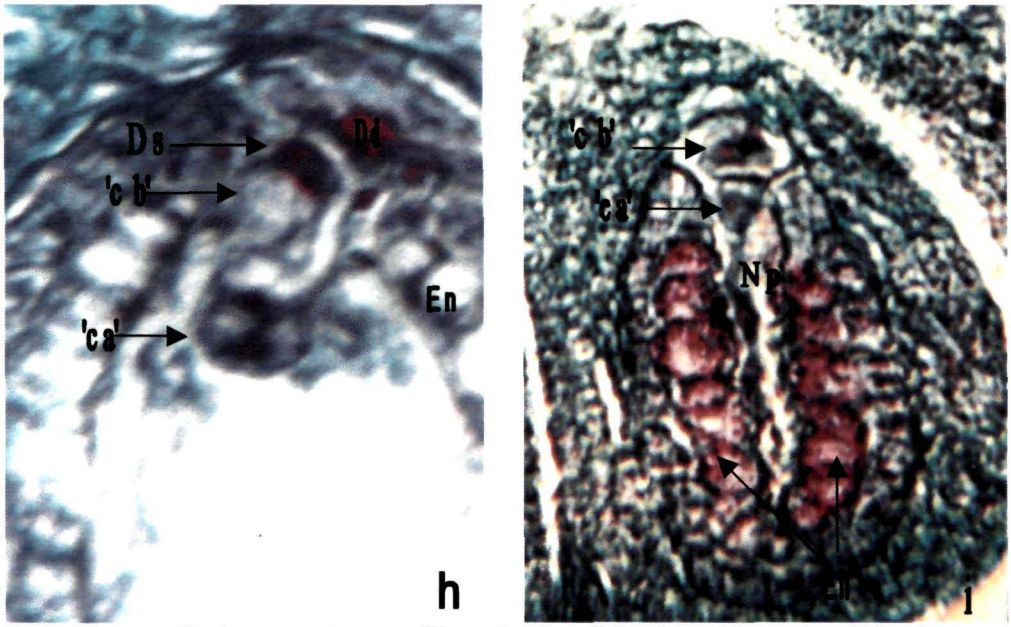
j Five celled, 4 - tiered proembryo. The distal cell (n') enlarged and different1iates into haustorium. Note the density of stain in 'ca' and 'm' and degenerated synergid (Ds) whereas n' takes less stain.

k Tetrad proembryo with quadrant (q) and the cells 'm' and 'ci'. Note the radially stretched endothelium (En).

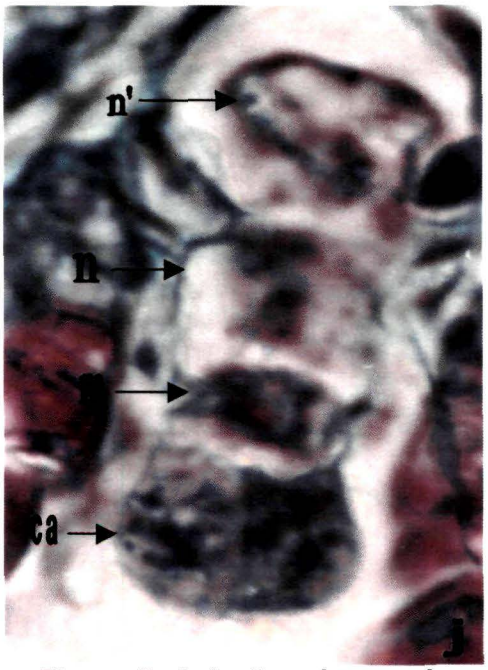
(Magnification- h& i= x 1150; j= x 1500; k= x 850)

Plate- 4.4

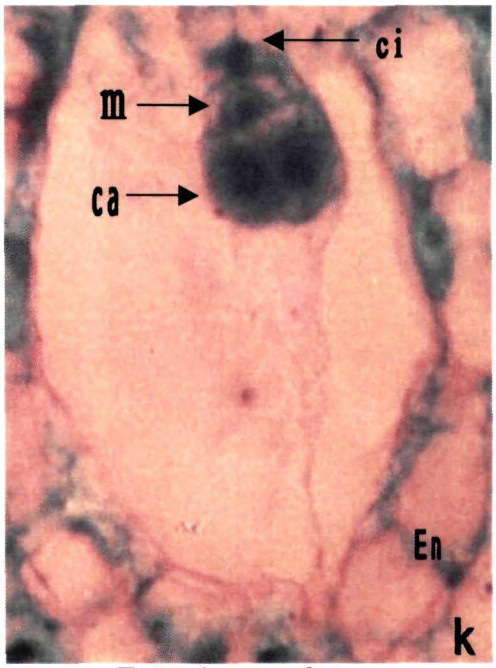
***Hydrobryum griffithii* (Wall. ex Griff.) Tul.**



Two celled-proembryo with apical cell (ca) and basal cell (cb).



Five celled, 4 - tiered proembryo



Tetrad proembryo

Plate- 4.4

l& m Octant proembryo (Oc) with suspensor haustorium (Sh) and a Globular pro embryo (Gl).

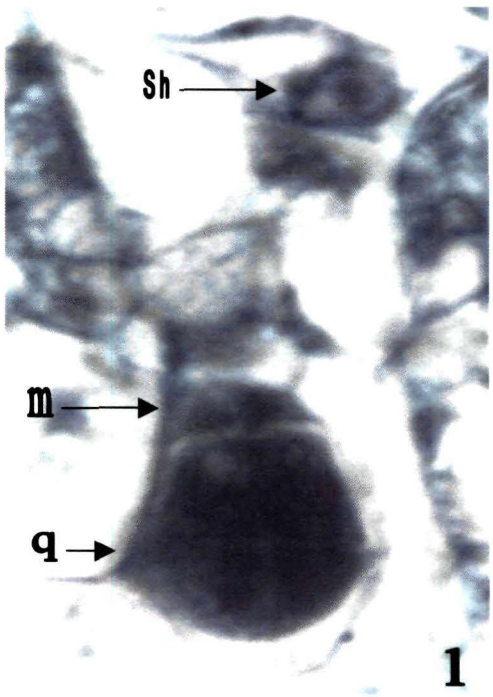
n Nucellar plasmodium (Np) with endothelium (En) and a part of embryo.

o Dissected matured embryo from the seed with cotyledons (Cot) and suspensor haustoria (Sh). Note the absence of shoot apex.

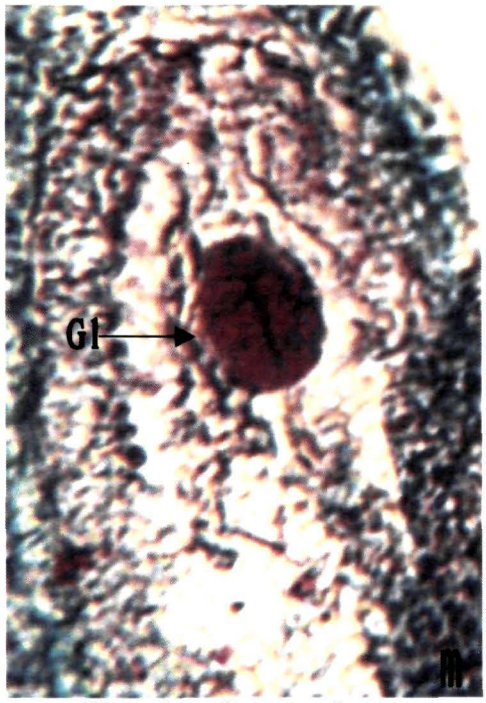
(Magnification- l& m= x 1000; n= x 800; o= x 700)

Plate- 4.4

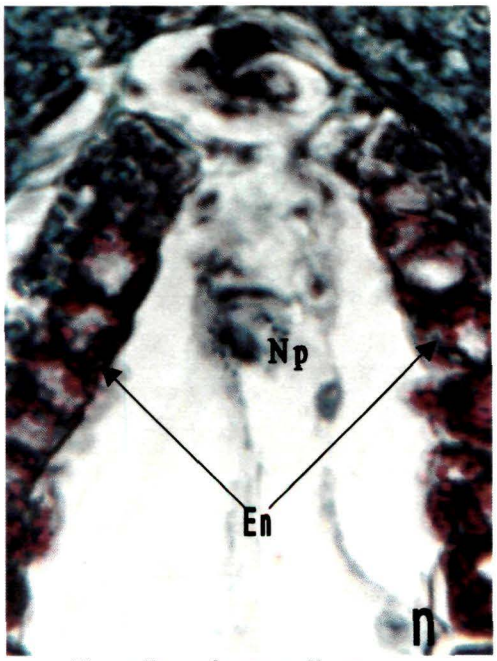
***Hydrobryum griffithii* (Wall. ex Griff.) Tul.**



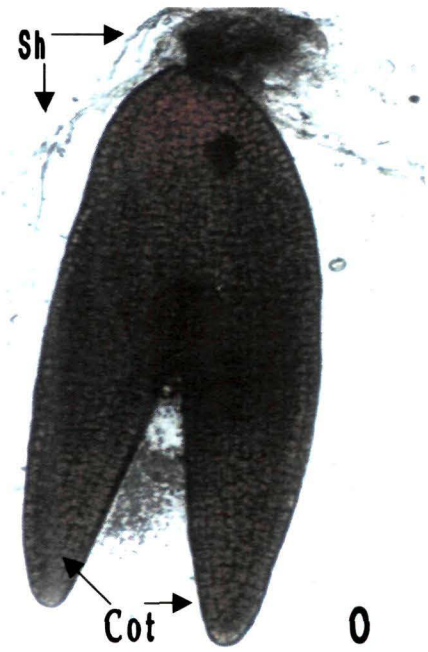
Octant proembryo with suspensor



Globular stage embryo



Nucellar plasmodium



Dissected mature embryo

CHAPTER – 5

Polypleurum wallichii

Polypleurum wallichii (R. Brown ex. Griff.) Warm. *Dicraea minor* (Bentham et Hooker) Weddell, D. *pterophylla* Weddell, *Polypleurum agharkarii* (Nandi) Nagendran, Arekal et Subramanyan nom. Illeg.Br. In Wall. Cat. 5225; *Griff.* In As. Research. xix. 103, t. 17, and Notul. 378, and *Ic.* Pl. Asiat. t. 541, f. 1, 542, 543; Gardn. In Calc. Journ. Nat. Hist. 183; Royle Ill. i. 331. *Dicraea Wallichi*, Tul. in Ann. Sc. Nat. Ser. 3, xi. 101, and Monogr. Podost. 118; Wight Ic. T. 1916, f. 1; Meissn. *In DC. Prodr.* xvii. 70. *Lacis Wallichi,* Steud. Nomencl. *Polypleurum orientale*, Tayl. Mss. *Blandovia striata*, Lehm. Mss.

5.1 Morphology and Habitat

5.1.1 Habit

The vegetative plant body is green and turn brownish at the start of reproductive phase; flat, bilaterally symmetrical, and fucoid with undulate margins (Plate 5.1d& e). It is fleshy, often branching exogenously and generally free drifting in the flowing water (Plate 5.1a). The conducting strand branches in conformity with the branching of the thallus. It is attached at the base by a stout

holdfast (Plate 5.1 j). Thallus various, usually free floating with marginal 1-flowered secondary shoot (Plate 5.1c).

Floral shoots developed at almost regular intervals of 2-3 mm along the upper edges of the margins bearing 3-6 scale-like, helmet shaped and keeled leaves (Plate 5.1f). Leaves caducous, thallus attached to the substrate at the base only, the rest free (Plate 5.1j).

Flowers zygomorphic, bisexual and hypogynous, buds usually long (Plate 5.1i). Young flower bud is enclosed by a thin transparent membranous, sac- like spathe which in turn is surrounded by bases of fleshy bract. Spathe 4 mm long, splitting irregularly at the tip, spathella opening into 4 lobes at the apex, about 2.5 mm long covering the pedicel (Plate 5.1f & g). There is branched stamen on the ventral side and two transparent cylindrical staminodes of about 5 mm long. Staminal filament is forked; each fork is bearing four loculed anthers, light brown to yellow in colour (Plate 5.1h).

Ovary sessile, bicarpellary, syncarpous and elliptic, green with brown longitudinal ridges (Plate 5.1h); smooth when young, ripening into capsule, 8 ribbed; 5-7 mm long and 3 mm broad; pedicellate, pedicels 4-5 mm, elongating into 8- 25 mm in fruit (Plate 5.1i). Two sessile stigmatic lobes are fleshy, subulate and dark brown in colour; slightly flattened with irregular surfaces.

Fruit a capsule, isolobous (Plate 5.1h). Flowering and reproduction takes place aerially only when the water level recedes (Plate 5.1b & c). The season starts in early September and fruiting observed in late October; seeds dispersal at the middle of November.
At the end of the season, the dry thalli are seen on the barren rocks (Plate 5.1k)

There are numerous minute ovules arranged in a greatly swollen axile placenta. Seeds bulbous, minute; testa membranous and transparent, tegmen dark brown in colour; length varies from 300µ- 350µ, breadth ranges from 180µ- 220µ, many (Plate 5.1 l& m).

5.1. 2 *Locality*

Specimen collection was carried out in stream at fossil park, Janiaw, in Lawbah region (Plate 5.1a& k); about 6 km away from Mawsynram, located in East Khasi Hills District, Meghalaya State, India (91° 50'N – 25° 20'E).

5.1.3 Key characters for *identification*

1. Cotyledons two.

   -Dicotyledons

2. Fruits capsule. Plants attached to rocks by disc-like holdfast with secondary branches; appearing like algae or bryophytes.

   -Podostemaceae

3. Perianth of 2, rarely 3 scales. Stamen 1 or 2-3 with filaments connate.

   -Eupodostemaceae

4. Flower zygomorhic, naked; the young buds covered or encircled by spathe.

   -Polypleurum

5. Thallus attached to substrate at the base only, the rest free. Pedicels as long as 5 cm when the fruit gets matured.

   P. wallichii

Plate- 5.1

a The habitat in Janiaw river. See the luxurious growh of plant in the torrent of river water.

b& c A part of plant community. As the water level recedes, the plant started flowering and blooms.

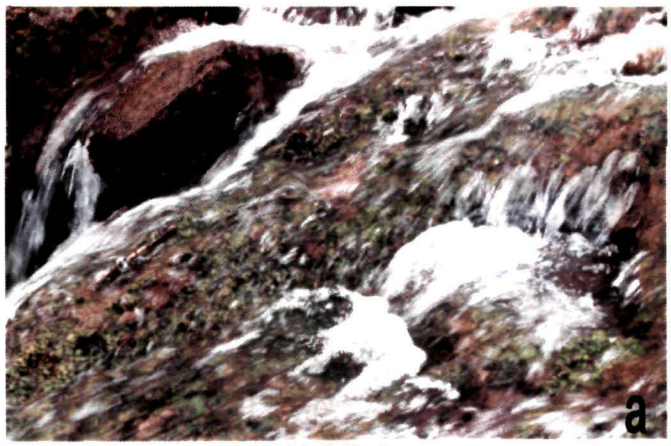
d& e Dorsiventrally flat thallus with filifirm leaves (L) on the margins.

(Magnification- b= x $^{1}/_{10}$; c& d= x $^{1}/_{2}$; e= x 4)

Plate- 5.1

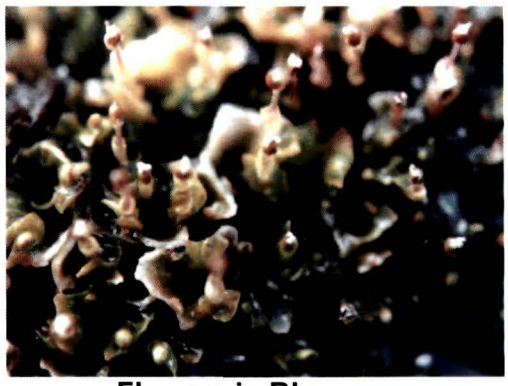
***Polypleurum wallichii* (R. Brown ex Griff.) Warm.**

Morphology and Habitat

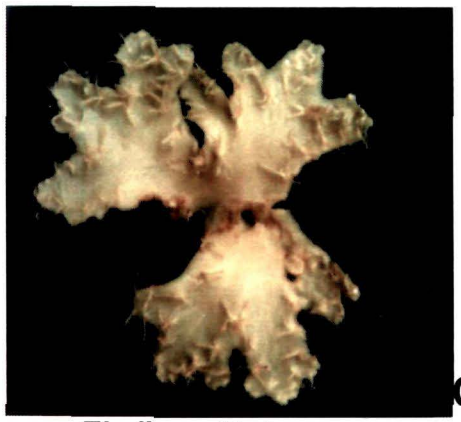


Habitat

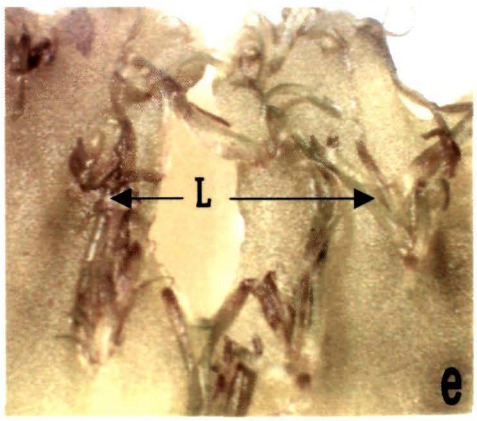
A Part of Plant Community



Flowers in Bloom



Thallus with leaves



A portion of thallus

Plate- 5.1

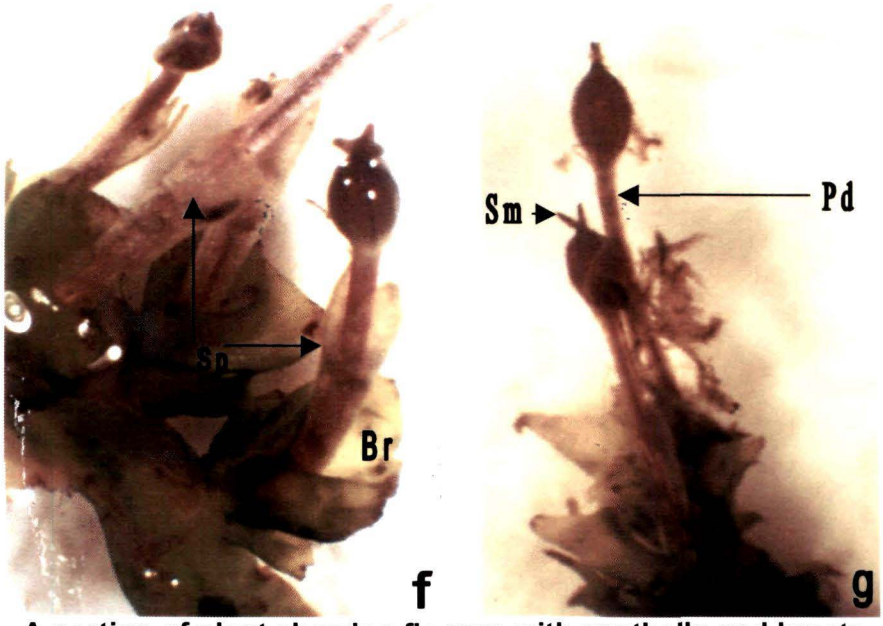
f& g A portion of plant showing flowers bifid stigma (Sm) in a pedicel (Pd) with spathella (Sp) and bracts (Br).

h& i An enlarged view of monoecious bisexual flower in light microscopy and SEM. See the longitudinal ridges in the ovary (O), bifid stigma (Sm), staminodes (St) and bilobed stamens (S).

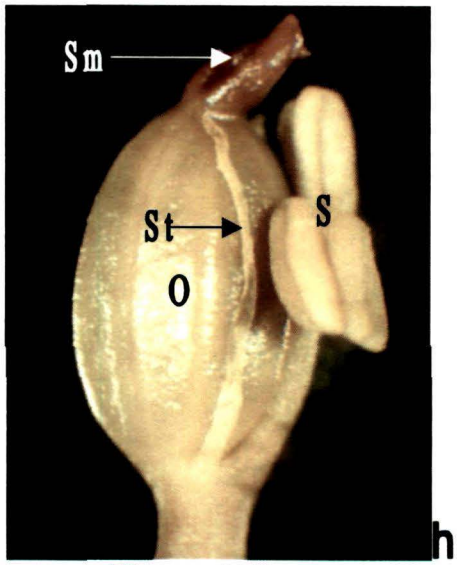
(f& g= x 5; h& I= x 15)

Plate- 5.1

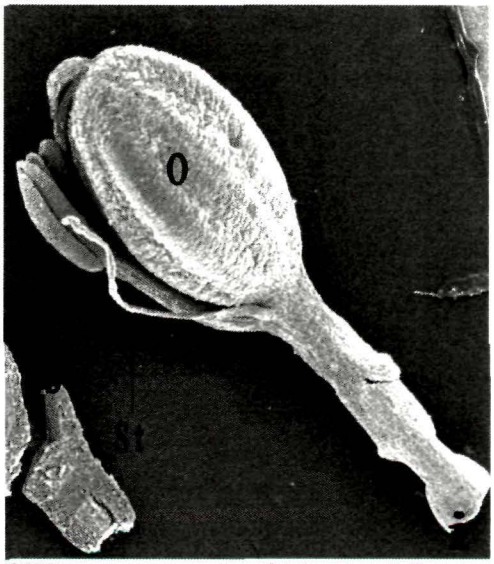
***Polypleurum wallichii* (R. Brown ex Griff.) Warm.**



A portion of plant showing flowers with spathella and bracts



Bisexual Flower



SEM micrograph of bisexual flower

Plate- 5.1

j On the underside of the thallus are unique holdfast called Haptera (H).

k At the end of monsoon period, a forest of Podoatemaceae are seen on barren rocks in the riverside.

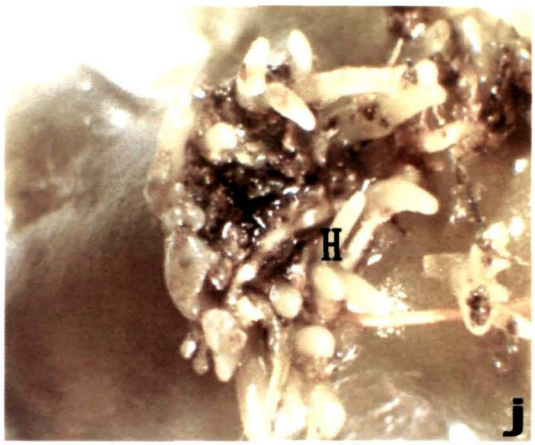
l SEM of longitudinally ribbed (Lr) mature capsule showing seeds (Se). Note the dehiscence of capsule is septicidal.

m SEM of a single seed. Note the ridges and grooves in surface.

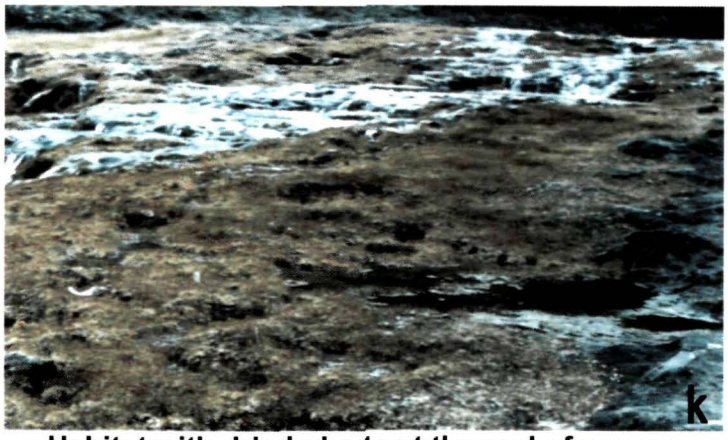
(j= x 15)

Plate- 5.1

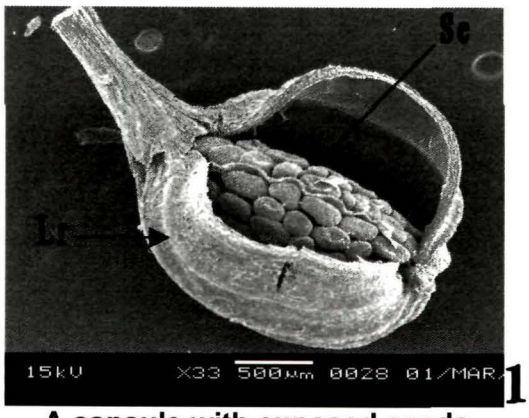
Polypleurum wallichii **(R. Brown ex Griff.) Warm.**



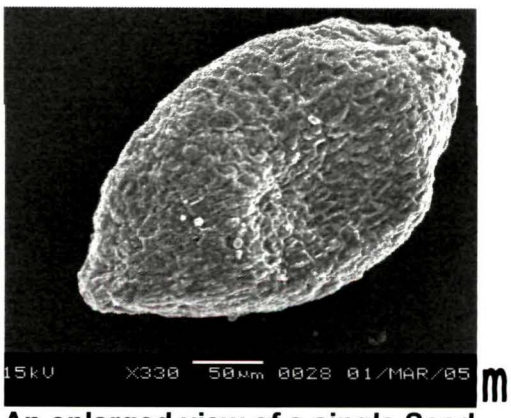
Holdfast or Haptera at the base of thallus



Habitat with dried plants at the end of season



A capsule with exposed seeds



An enlarged view of a single Seed

5.2 Anther Development and microsporogenesis

5.2.1 *Initiation of Microsporangium*

The stamens are bifid, each lobe contains tetrasporangiate anther (Plate 5.2a,d& s). The two stamen primordia developed prior to gynoecium. On initiation, the staminal primordium comprises a mass of undifferentiated meristematic cells and each primordium comprises of unilayered dermatogen that covers the multicellular hump-like tissues (Plate 5.2b). This configuration conforms to tunica-corpus concept. Ontogenenetically the bifid stamens are two independent units, because of intercalary growth at the base of stamen (andropodium) the filament becomes biforked or bifid (Plate 5.2c& e) and each fork bearing a single tetrasporangiate anther lobe. Each stamen primordium after reaching 15 μm in length, the vasculature differentiates in acropetal direction (Plate 5.2b). The anther primordia are differentiated on the adaxial side; so that, all the four lobes of each anther facing towards the stigma (Plate 5.2d).

5.2.2 *Archesporial cell and Anther wall formation*

A plate of 3-4 hypodermal cells differentiated into an archesporium in each anther lobe. Archesporial cells are radially elongated, densely cytoplasmic with a large, more prominent nucleus (Plate 5.2f). The entire archesporial initials divide

periclinally, to form two layers that are of unequal in size; the inner row of cells is much larger than the outer ones. The former becomes primary sporogenous cell and the latter one primary parietal cells. The primary sporogenous cell divide and re-divides to form sporogenous tissue (Plate 5.2e).

The primary parietal cells undergo only one periclinal division to form two-layered secondary parietal layer, which directly transformed into endothecium and tapetum respectively, the middle layers are absent (Plate 5.2 g). Therefore the ontogenic development of anther wall layer belongs to reduced type. The cells of epidermis, endothecium and tapetum are fully laddened with starch grains. The starch grains in the endothecial layer start to disappear when the pollen mother cells enter into meiotic prophase-I. The endothecial layer as well as adjacent to the connective tissue also has fibrous annular thickenings that develop at the time of meiosis-II in the meiocytes (Plate 5.2 l).

5.2.3 *Tapetum*

Tapetum is secretory, unilayered in structure enclosing the sporogenous tissues. The cells are rectangular, both uni and bi-nucleate condition cells are seen (Plate 5.2 g). The cells are densely cytoplasmic with lots of starch and protein contents. The entire tapetum is derived from inner layer of the secondary parietal layer. The tapetum releases granular substances that are similar

to that of sporopollenin deposited on the inner tangential wall, facing towards the anther locule (Plate 5.2q). Under light microscope, these granular substances appear in the tapetal cells at the end of meiosis-I (Plate 5.2 r). The tapetal cells persist until the release of pollen grains in pairs.

5.2.4 *Meiosis and Cytokinesis*

Prior to meiosis, the microspore mother cells becomes rounded and separated out (Plate 5.2 g). The microspore mother cells are enclosed by a distinct callose wall that gets dissolved after the completion of meiosis-II and before the onset of pollen wall deposition. Meiosis is of successive type. At the time of prophase-I, the chromosomes are distinctly organized as minute rods in the prophase nucleus (Plate 5.2 h), followed by metaphase with nuclear spindles at the equatorial plane (Plate 5.2 i& j) and a dyad is formed (Plate 5.2 k& l). After the first meiotic division a distinct cell plate is formed and the nuclear spindle disappeared. As soon as the first meiotic division is over, the nuclei of daughter cells immediately undergo second meiotic division (homeotypic division) without any resting period in which the spindles of metaphase-II are either parallel or right angles to each other. A tetrad resulted in second meiotic division (Plate 5.2m), which then get separated and the pollen grain are dispersed in pairs (Plate - 5.2n).

During and after meiosis-II, the pro-orbicules (Ubisch bodies) are released from tapetum and deposited on the pollen exine (Plate 5.2 r). Within the initial stages of microspore enlargement, the cytoplasm does not increase rapidly enough to fill the entire lumen of the microspore wall. Cytoplasmic vacoulation increases and when the microspores enlarge to their maximum size, the cytoplasm forms only a thin layer around nucleus with radiating strands. Subsequently the cytoplasm increases quickly and the nucleus shifts its position to the distal pole where it divides to form the vegetative and generative cells (Plate 5.2 n). Because of the callose wall, pollen grains fluoresce brightly in fluorescence microscope (Plate 5.2o). Pollen germination on the stigmatic surface in natural condition has also been observed (Plate- 5.2t).

5.2.5 *Pollen grains*

Pollen grains of *Polypleurum* occur in dyads. Dyads average 22.5µm and 9.8µm in width (Plate 5.2 q). The shared wall between the two grains averages 5-7 µm in width. Pollen grains are arranged in isobilateral or decussate arrangement (Plate 5.2 m& n). Individual grains of the dyad are spherical minutely granulate with with small protuberance on the side (Plate 5.2 r). Exine 1 µm thick; microechinate ornamentation characterizes the pollen surface. Microechinate processes from 0.2-0.4 µm large in height,

the distribution of the processes is regular and density is three micoprocesses per μm^2. Ornamentation concentrically arranged at the poles. Pollen kitt- abundant.

5.2.6 *Pollen viability*

The pollen viability of *Polypleurum wallichii* was studied using Flourochromatic Reaction (FCR) Test. The viable pollens fluoresce brightly and non-viable ones did not fluoresce (Plate 5.2 p). *Polypleurum wallichii* shows high pollen viability percentage. The FCR test revealed that 82.60% of pollen grains were viable.

Table – 5.2.6: Pollen viability test by using FCR Test

Name of Plants	Total no. of pollens		No. of viable pollens		Percentage of viability
Polypleurum wallichii (R. Brown ex. Griff.)	R_1	205	R_1	191	82.60 ± 8.12
	R_2	63	R_2	55	
	R_3	267	R_3	176	
	R_4	154	R_4	140	
	R_5	87	R_5	79	
	Mean	155.2	**Mean**	128.2	

± : Standard deviation (SD)

R_X: Number of replicates

Plate- 5.2

a SEM micrograph of stamen with bilobed anthers (An). See the forked filament (F).

b L. S. of young flower showing two stamen primordia (Sp) to exhibit a vasculature (V) enclosed within a perianth (Pr).

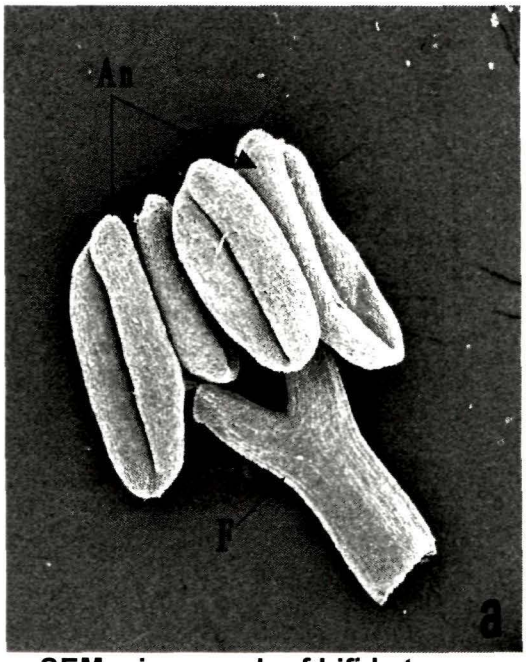
c L. S of flower showing intercalary growth in the andropodium (Ap) with biforked filament (F) and anthers (An) covered within a perianth (Pr).

(a= x 30; b= x 200; c= x50)

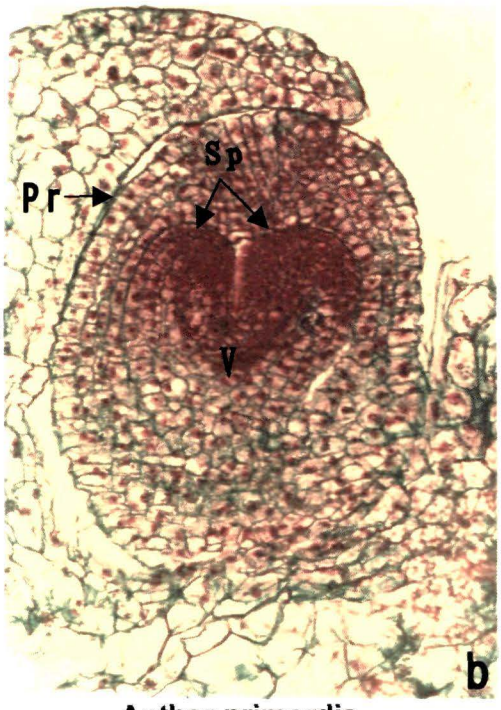
Plate- 5.2

Polypleurum wallichii (R. Brown ex Griff.) Warm.

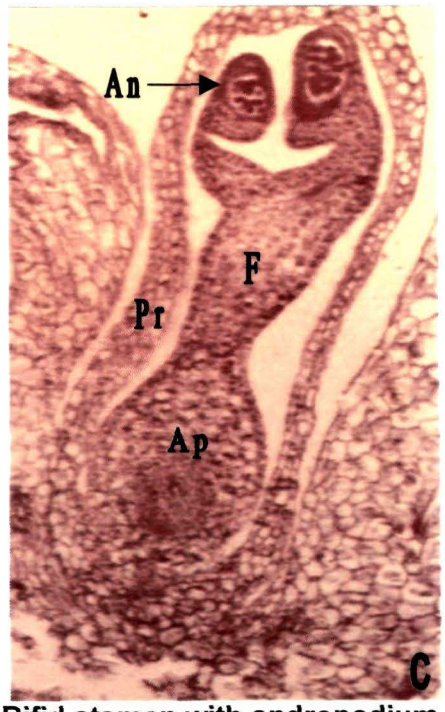
Anther Development and Microsporogenesis



SEM micrograph of bifid stamen



Anther primordia



Bifid stamen with andropodium

Plate- 5.2

d L. S of young flower showing a sporogenous tissues with tetra-locular anthers (An) facing towards the stigma.

e Anther locules (An) facing each other.

f An enlarged view of young anther lobe showing a row of hypodermal archesporial initial (Ai) below the epidermis (E).

g Microspore mother cells (mmc) inside the wall layers, tapetum (Tp), endothecium (Em) and outermost epidermis (E).

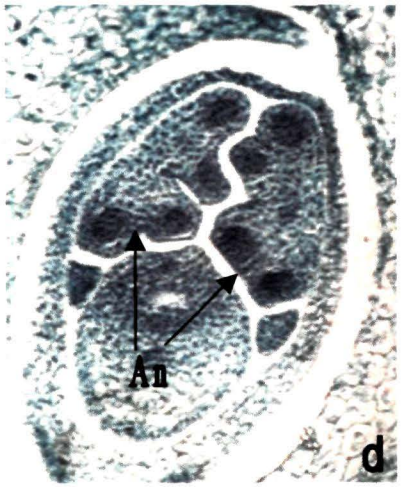
h Prophase nucleus of meiosis-I in microspore mother cell with rod like chromosome (Cr).

i Metaphage stage of meiosis-I in micospore mother cell.

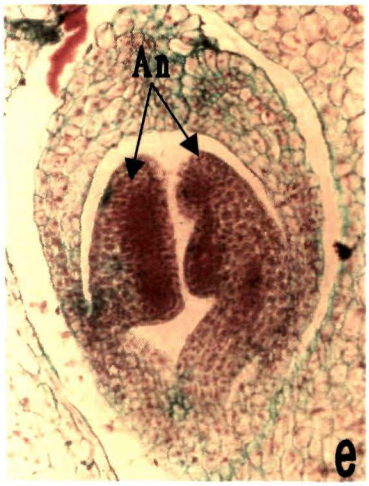
(d= x 150; e= x 200; f= x 350; g= x 160; h= x 1000; I= x300)

Plate- 5.2

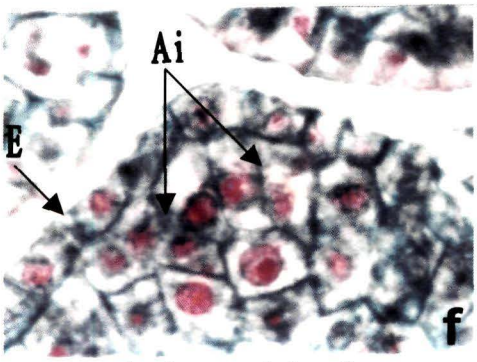
Polypleurum wallichii (R. Brown ex Griff.) Warm.



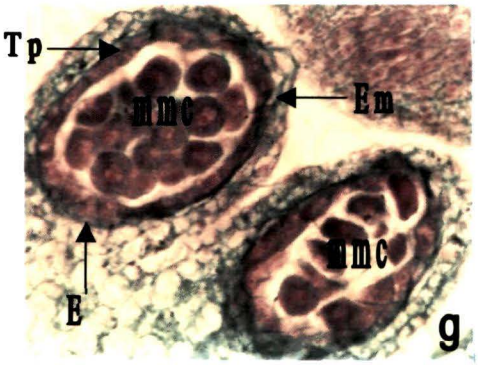
T.S. of young flower



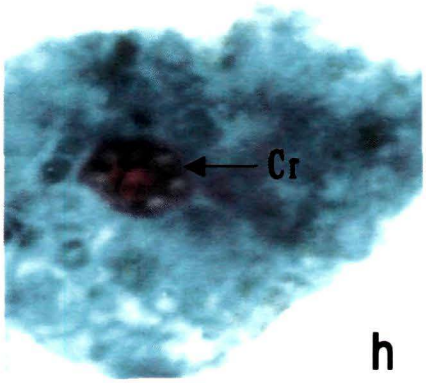
L.S of flower with young anthers



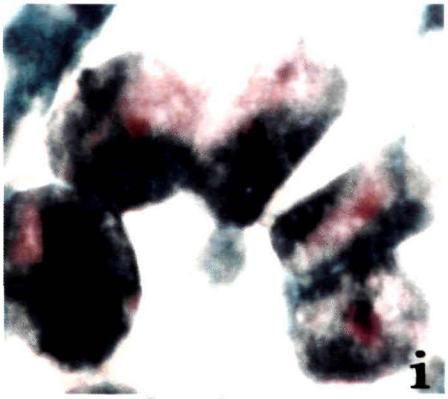
Archesporial cells



Microspore mother cells



A Prophase nucleus



Metaphase

Plate- 5.2

j Another view of metaphase-I with chromosomes in the equatorial plate.

k Telophase stage with dyad (D) formation.

l Dyads (D) with distinct cell plate enclosed within tapetum (Tp).

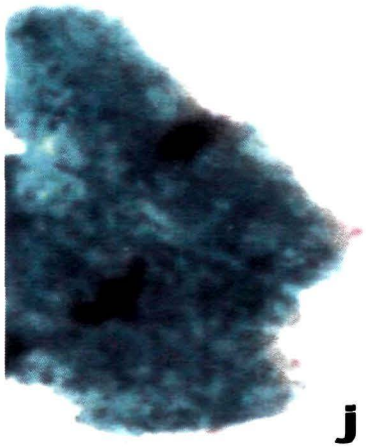
m& n Tetrad of meiosis- II separates and dispersed the pollen grain (PG) in pairs.

o The pollen grains give florescence with aniline blue because of the callose (Ca) deposits.

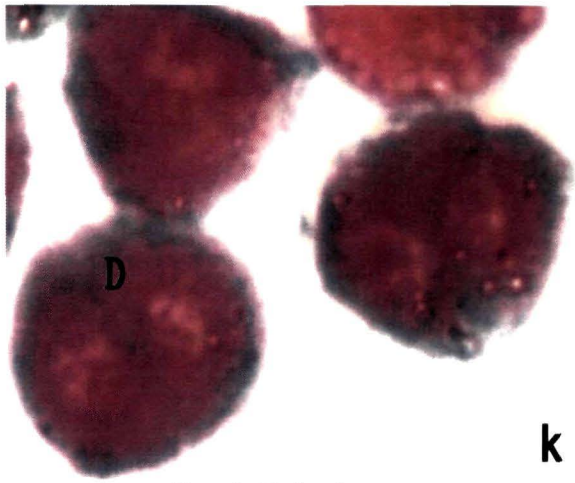
(j= x 600; k= x 520; l= x 500; m= x 400; n= x 300; o= x 100)

Plate- 5.2

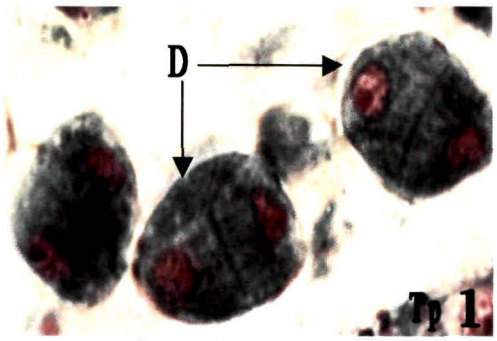
Polypleurum wallichii **(R. Brown ex Griff.) Warm.**



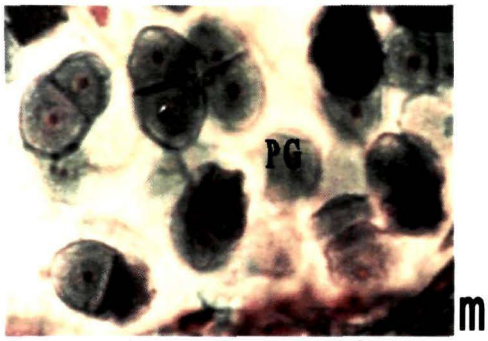
Meiosis –I, Metaphase



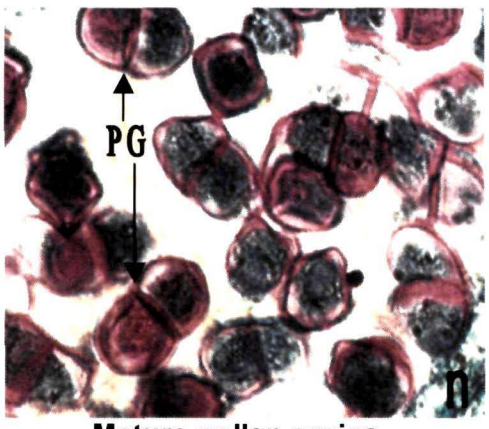
Dyad- Telophase



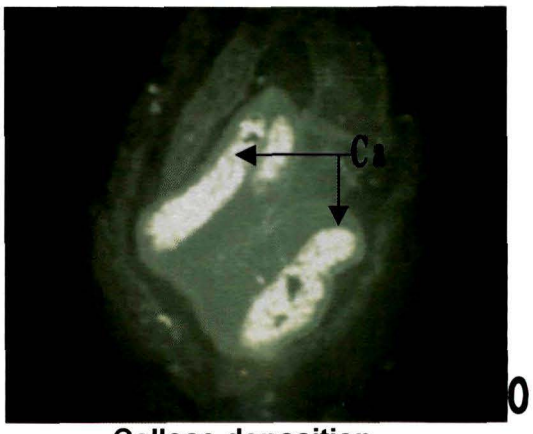
Dyad formation



Tetrad separated in pairs



Mature pollen grains



Callose deposition

Plate- 5.2

p Pollen viability test by FCR test. Only the viable pollens give fluorescence with FDA in fluorescence microscope.

q& r Dyad pollen grains (PG) in SEM. Note the hooked appearance at the distal end of pollen grains with sporopollenin deposits in a granular fashion. See the tapetum (Tp).

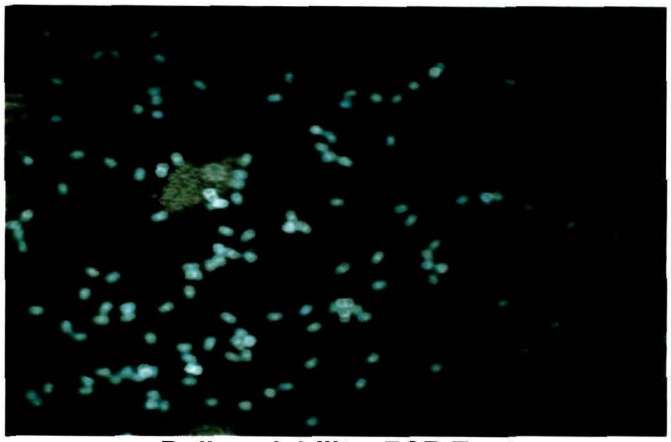
s Transverse section of mature flower showing tetralocular anthers (An) and isolobous ovary (O).

t Germination of pollens on the stigmatic surface (Sm) under natural condition observed in fluorescence microscope.

(p= x 200; s= x 100; t= x 350)

Plate- 5.2

Polypleurum wallichii **(R. Brown ex Griff.) Warm.**

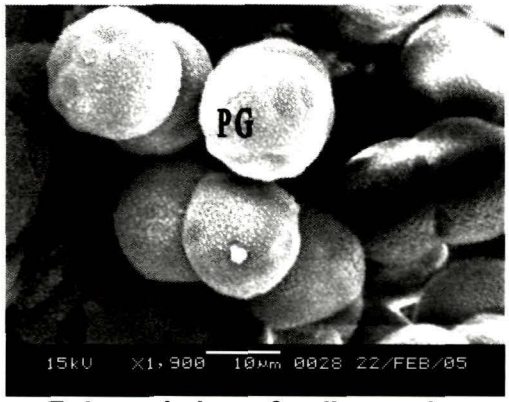


p

Pollen viability- FCR Test

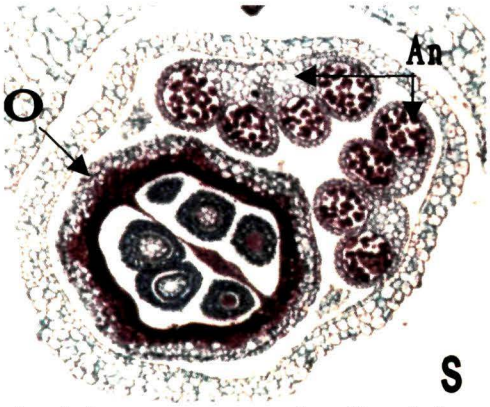
q

SEM micrograph of pollen grains



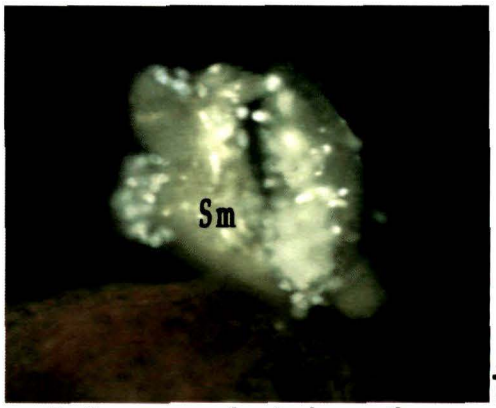
r

Enlarged view of pollen grains



S

Isolobous ovary and anther lobes



t

Pollens germinated on stigma

5.3 Megasporogenesis and Embryo sac development

5.3.1 *Megasporangium*

In *Polyplerum wallichii,* flowers are bisexual enclosed within a perianth also called spathella (Plate 5.3 c). Ovary is isolobous; SEM micrograph shows numerous ovules arranged on the thick swollen placenta (Plate 5.3 a& b).

The ovule primordium arises as a small protuberance consisting of a single cell surrounded by an outermost protoderm (Plate 5.3 d& g). The central cell grows in height by transverse divisions and at the same time the protodermal layer undergoes anticlinal divisions. When the central row of cells about four to five in number, the outer integument is initiated from the epidermal layer just four to five cells below the apex of the ovule primordium, as a result of which a tenuinucellate ovule with single integument is differentiated in the beginning (Plate 5.3 e). Just below the outer integument, i.e. the curvature of funiculus takes place on the convex side of the ovule primordium. So that the tenuinucellate ovule turns over 180° (Plate 5.3 f). The funicular growth on the concave side (raphal side) shows less development.

5.3.2 *Megasporogenesis*

In *Polypleurum Wallichii,* the hypodermal cell in tenuinucellate ovule directly functions as archesporial initial. As

soon as the archesporial initial is differentiated the initiation of inner integument takes place at the junction of the nucellus and chalaza (Plate 5.3 h). The further growth and development of inner integument pushes the nucellus along with archesporial cell towards the micropyle. Due to the activity of the inner integument, the archesporial chamber (I used the terminology archesporial initial and its envelope of a single layer of epidermal cells except the cell at the base as an archesporial chamber) is located in between the outer and inner integument towards the micropylar end (Plate 5.3 i).

Archesporial initial is comparatively larger in size with distinct nucleus, nucleolus and dense cytoplasm and directly functions as megaspore mother cell (Plate 5.3 j). During the onset of meiosis, protoplast is isolated from the epidermal layer by deposition of callose wall and the nucleus moves towards the micropylar end (Plate 5.3 k). The megaspore mother cell is radially elongated and a nuclear spindle appears in the equatorial plane (Plate 5.3 l). The first meiotic division result into the two-celled dyad, out of which the micropylar cell is comparatively larger than the chalazal one (Plate 5.3 m). The micropylar one eventually degenerates and disappear. The chalazal cell of the dyad only takes part in the meiosis-II, during which cell plate formation is absent. Therefore, a binucleate megaspore is formed. The

persistent degenerated micropylar cell is seen until the formation of bisporic megaspore (Plate 5.3 n).

5.3.3 *Embryo sac development (Megagametogenesis)*

In the bisporic megaspore, the two nuclei are located close to each other in the center of the functional megaspore. These two nuclei move towards their respective poles -i,e. micropylar and chalazal axis (Plate- 5.3 n). The micropylar and chalazal nuclei undergo mitotic division without cell plate formation. As a result, tetranucleate embryosac is formed (Plate 5.3 o). The following two types of embryosac organization are encountered in *Polypleurum wallichii*.

I- In the micropylar nucleus of the bisporic megaspore, spindle formation is horizontal, so that the resultant two nuclei are laid side by side or juxtaposed; whereas in the chalazal nuclear division the spindle formation is transverse as a result of which the two nuclei are laid one above the other. In this tetranucleate embryosac, two nuclei are at the micropylar pole; one nucleus at the chalazal pole and the fourth one (egg nucleus), which is larger than the rest, lie in between them. The central nucleus thus functions as egg cell/ egg nucleus (Plate 5.3 p). The two at the micropylar pole converted into two

synergids, while the chalazal nucleus transformed into polar nucleus.

II- The second type is just the reverse of the previous one. The chalazal nuclear spindle is horizontal; on the other hand, the micropylar one is transverse. So that one nucleus located in the micropylar end, two nuclei lie side by side in the chalazal end, the fourth nuclei in the center (Plate 5.3q & r). In this case, the two synergids are located at the chalazal end, the polar nucleus at the micropylar end; the centrally located nucleus is the functional egg cell.

Therefore, two types of embryosac formation, i.e. *Polypleurum* type and *Podostemum* type have been observed in P. *wallichii*. Whatever may be the type of organization of embryosac, the central nucleus or cell always functions as egg in both the cases.

Plate- 5.3

a& b SEM micrographs of mature ovary in Transverse and longitudinal plane. See the ovules (O) arranged on the thick placenta (Pl). The placentation is axile.

c Gynoecium (G), stigma (Sm) and one anther locule (An)

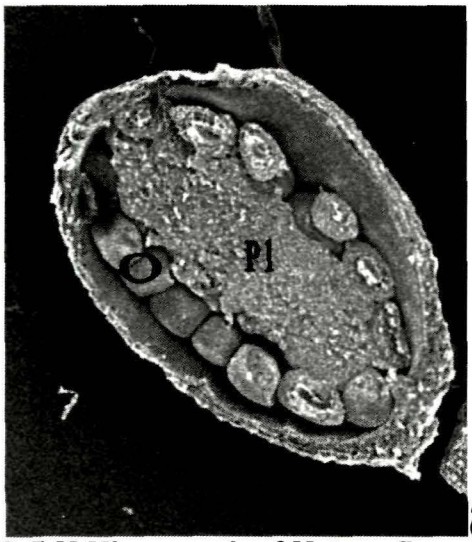
d L. S of young ovary showing ovule primordia (Op) in the placenta (Pl).

(c= x 300; d= x 35)

Plate- 5.3

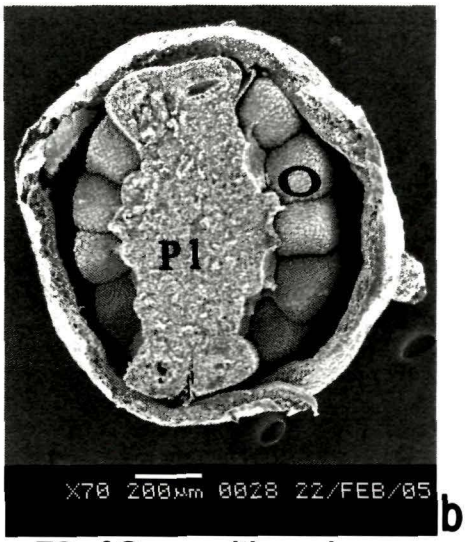
Polypleurum wallichii (R. Brown ex Griff.) Warm.

Megasporogenesis and Embryo sac formation



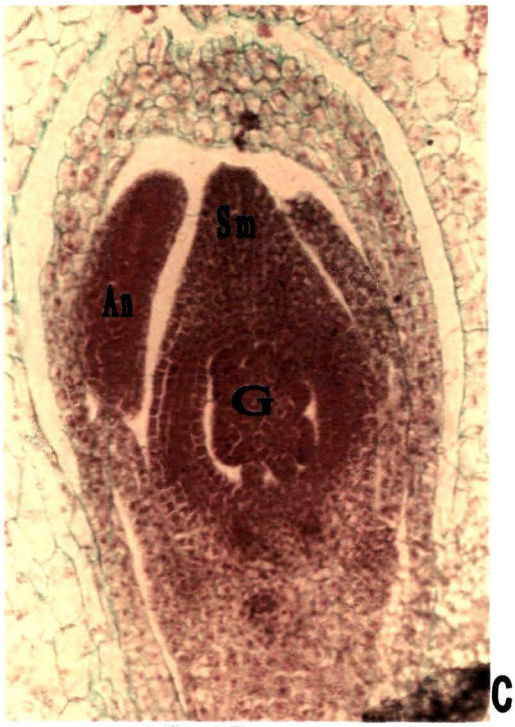
a

S.E.M Micrograph of Mature flower



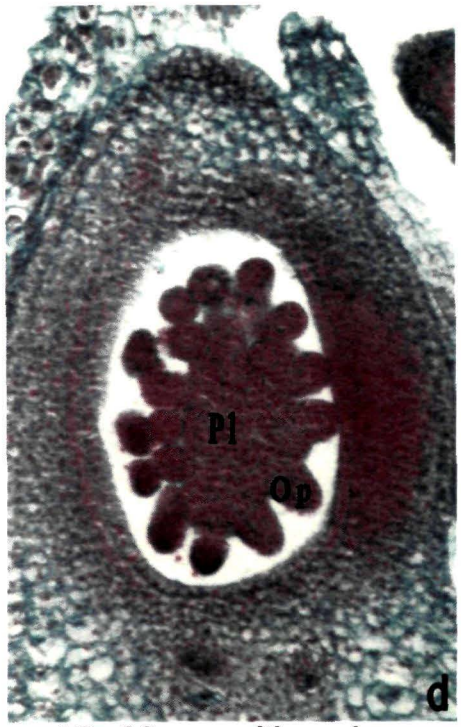
b

TS of Ovary with ovules



c

LS of flower



d

LS of flower with ovules

Plate- 5.3

e Anatropous ovule showing the nucellus (Nu) outer integument (Oi) and a funicle (Fu).

f A single ovule initiated with nucellar cells (Nu).

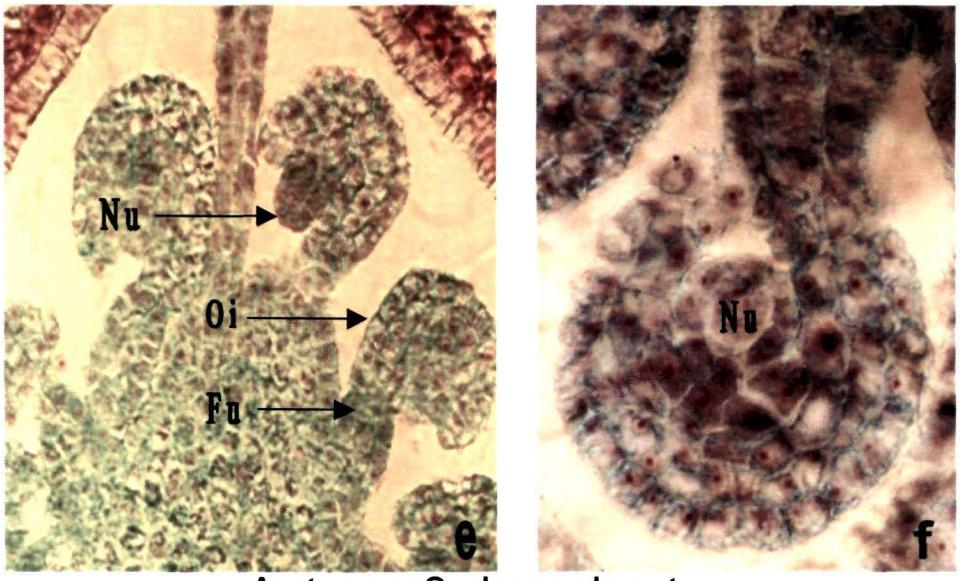
g An enlarged view of ovule primordium showing nucellar epidermis (nE) and nucellus (Nu).

h Archesporial initial (Ai) initiated. See the development of inner integument (Ii).

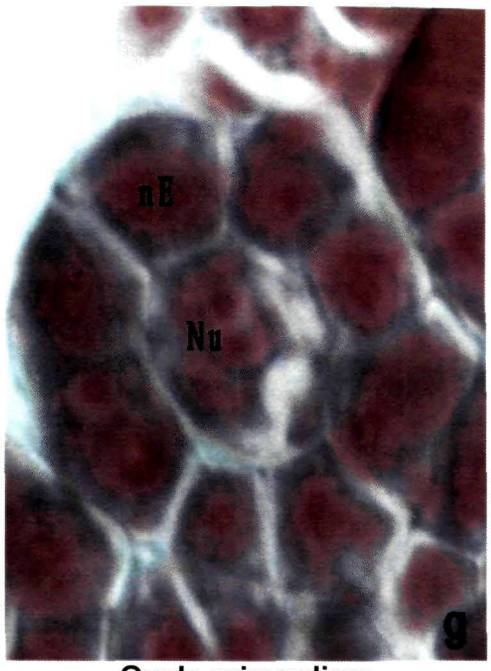
(e= x 350; f= x 670; g= x 900; h= x 1400)

Plate- 5.3

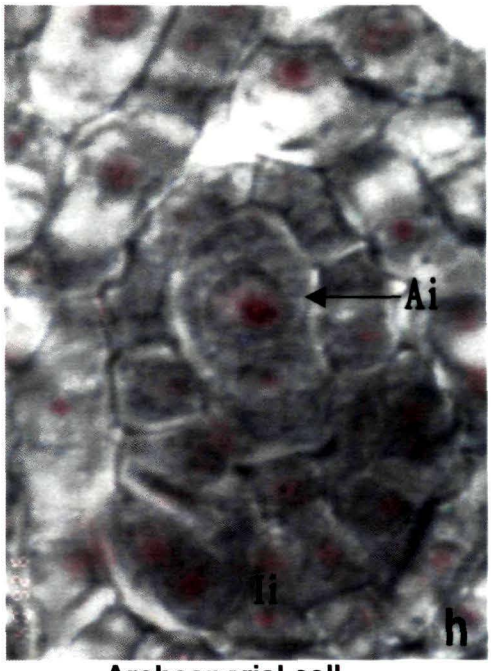
***Polypleurum wallichii* (R. Brown ex Griff.) Warm.**



Anatropous Ovules on placenta



Ovule primordium



Archesporial cell

Plate- 5.3

i Archesporial initial directly fuctions as megaspore mother cell (MMC) differentiated. See the tenuinucellate condition (Nu) of the ovule.

j Megaspore mother cell (MMC) differentiates in having dense cytoplasm and large central nucleus.

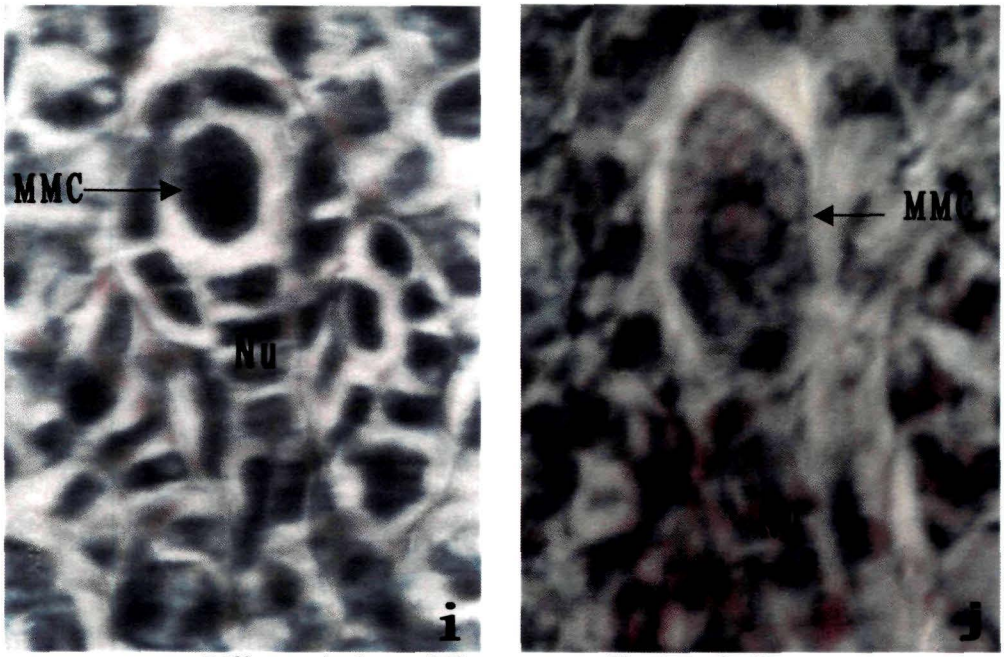
k The nucleus develops distinct nucleolus and moves to the micropylar end.

l Meiosis- I take place in MMC. The nuclear spindle (Ns) is visible in the equatorial plate.

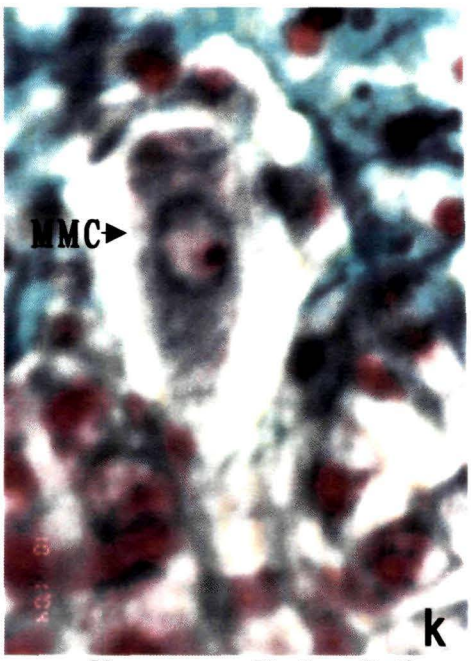
(i= x 860; j= x 1800; k& l= x 1650)

Plate- 5.3

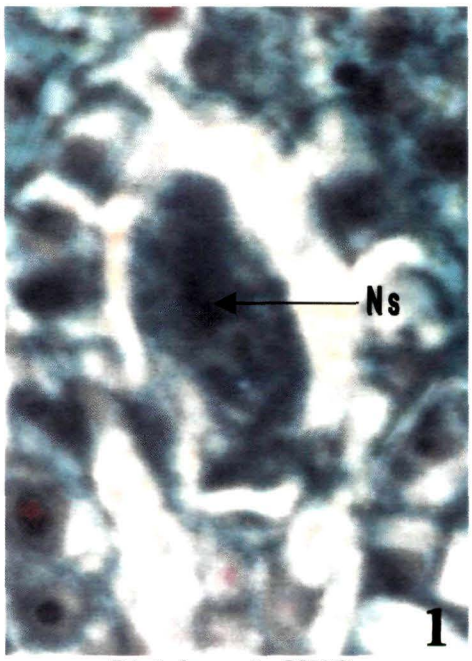
Polypleurum wallichii (R. Brown ex Griff.) Warm.



Differentiation of Megaspore Mother Cell



Megaspore Mother Cell



Division of MMC

Plate- 5.3

m Dyad (D), often called micropylar dyad is formed of meiosis- I.

n The micropylar nucleus degenerated (Dd) and the chalazal one divides to form two nucleate functional megaspore (Fm) conforming to bisporic development of embryo sac.

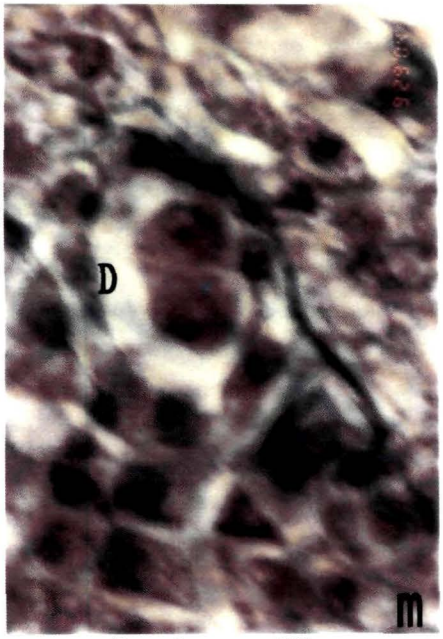
o Only one cycle of mitosis takes place to form four nucleate embryo sac, Egg apparatus (Eg) comprises of two synergids and egg cell.

p L. S of ovule showing degenerated dyad (Dd) at the micropylar end; embryo sac consist of two synergida (Ss), egg cell (Ec) and polar nucleus (Pn).

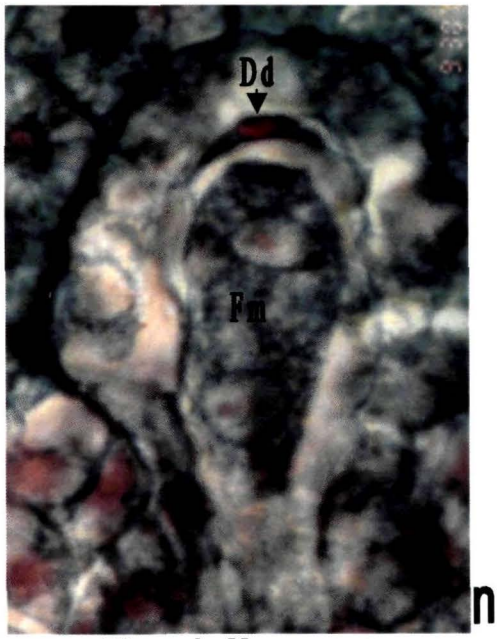
(m& n= x 1600; o& p= x 1450)

Plate- 5.3

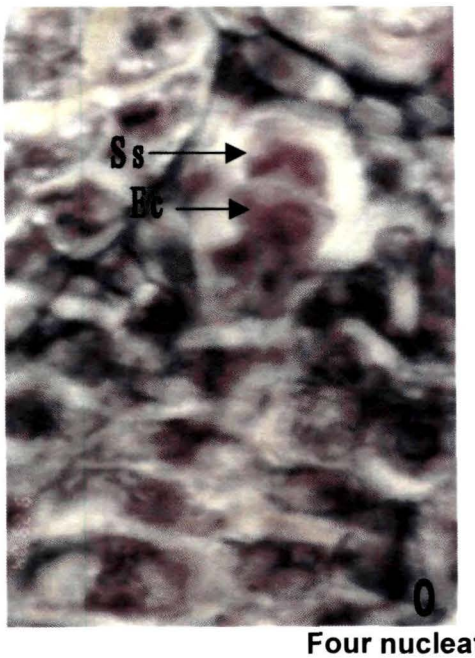
***Polypleurum wallichii* (R. Brown ex Griff.) Warm.**

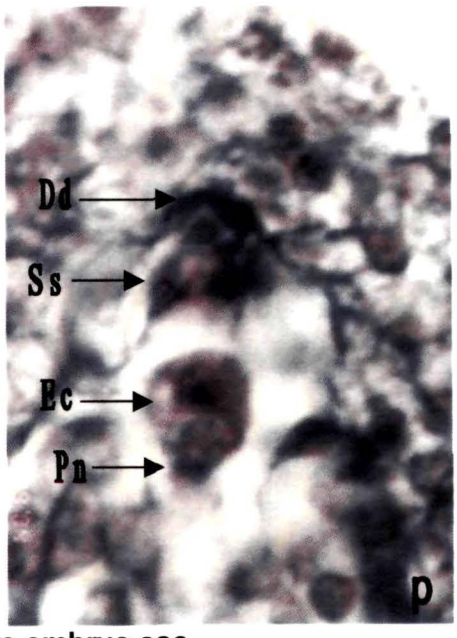


Dyad condition



Bisporic Megaspore





Four nucleate embryo sac

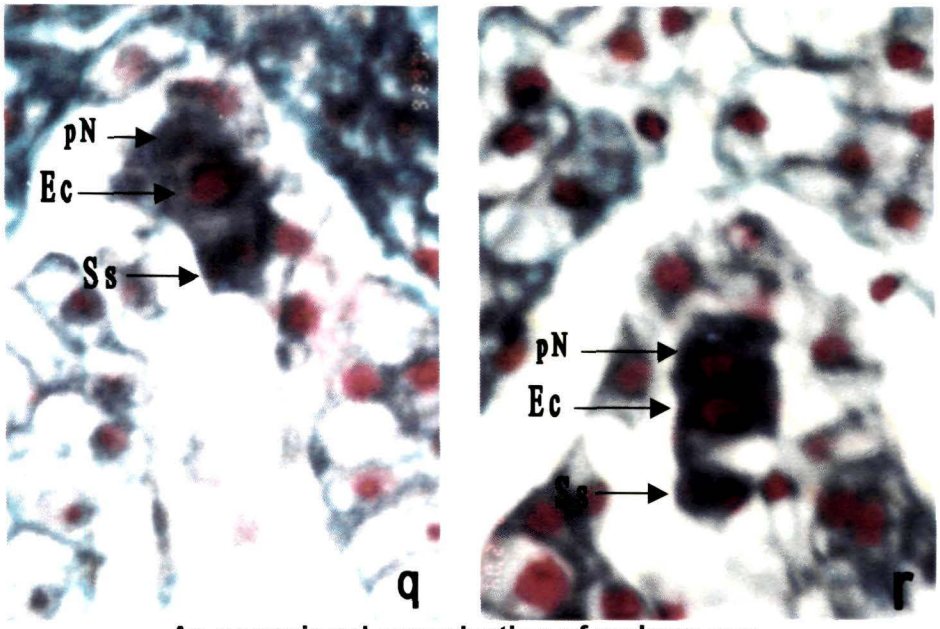
Plate- 5.3

q& r L. S of flower showing dicraea type of embryo sac formation. Note the synergids (Ss) occupied chalazal pole with egg cell (Ec) and micropylar polar nucleus (pN).

(q= x 1600; r= x 1550)

Plate- 5.3

***Polypleurum wallichii* (R. Brown ex Griff.) Warm.**



An occasional organization of embryo sac

5.4 Embryogeny

5.4.1 *Zygote*

The fertilized egg or zygote has a large nucleus with dense cytoplasms (Plate 5.4 a); because of its increasing volume, pushed the polar cell toward the chalazal end. The degenerated polar nucleus can be noticed. The entire protoplast of the zygote is enclosed by a thin cellulosic cell wall. The zygote occupies a major portion of the embryo sac. The disintegrated synergids and the dyad cell persist as dark structures (Plate 5.4b).

5.4.2 *Embryogenesis*

In *P. Wallichii*, the first division of zygote is in transverse plane, thus giving rise to a superposed arrangement of the daughter cells (Plate 5.4 d). Of these, the cell that is nearest to the micropyle is the basal cell (Conventionally designated as 'cb') and the one that is away from the micropyle is the terminal call (Conventionally designated as 'ca'), the degenerated dyad cell and degenerated synergid are observed. During the division these cells the wall is laid down in transverse plane. The basal cell divides to form 'm' and 'ci'; of which 'ci' is located more basally and the 'm' is placed in between ca and ci. (i.e.- an intermediate position) to form a 4-celled linear filamentous pro embryo (Plate 5.4 e). The apical cell divides longitudinally so that a T-shaped

tetrad is formed in which the daughter cells of the terminal tier are adjacently placed in a single tier (Plate 5.4 f).

The adjacently placed cells divide by a vertical wall at right angle to the previous partition, thus giving rise to a four-celled structure in two tiers (Plate 5.4 g). These four cells undergo the next division by transverse division, thereby producing the octant proembryo. The component cells of octant stage became disposed in two superposed tiers (I and I^{I}) of four cells each (Plate 5.4 h).

The octant stage is followed by numerous cell divisions in various planes causing the pro embryo to assume a globular configuration. The cotyledonary loci and epiphyseal locus are differentiated from the globular proembryo (Plate 5.4 i). The cotyledonary loci develop much faster than the hypophyseal region. As a result, a dicotyledonous embryo is differentiated within the ovule (Plate 5.4 j&k). A mature embryo consists of two cotyledons with wavy thread like suspensor haustoria (Plate 5.4 l). The embryo is devoid of plumule and radicular portion (Plate 5.4 m). Rhizoids develop from hypocotyls as a characteristic of the family (Plate 5.4 n). The cotyledons grow and emerge out of the seed coat (tegmen) (Plate 5.4 o).

5.4.3 *Suspensor Haustorium*

The development of suspensor is much faster than the differentiation of embryo. When the proembryo reaches the octant stage, the differentiation of suspensor is almost completed. The suspensor develops from the basal cell 'cb'. It divides transversely to form 'm' and 'ci' (Plate 5.4 e). The cell 'm' divides vertically to form juxtaposed cells. Whereas the cell 'ci' divides transversely 1 or 2 times to form the linear suspensor.

The uppermost cell of the suspensor (n') divides vertically to form two cells lying side by side, which have been transformed into suspensor haustoria just below the micropyle (Plate 5.4 f). The nuclei of haustorial cells became hypertrophied (Plate 5.4 g).

5.4.4 *Nucellar Plasmodium*

The nucellar cells situated below the megaspore mother cell enlarge considerably, especially in the longitudinal direction (Plate 5.4 c). Their walls become extremely thin and stain lightly that they are likely to be overlooked in most preparations. The pseudo-embryo sac is fully formed much before the embryosac is organized, most probably at early meiosis.

5.4.5 *Endothelium*

The inner integument is initiated after the complete development of the outer integument. The inner integument

consists of two layers of cells, out of which the inner layer adjoining with nucellar plasmodium is transformed into integumentary tapetum, which is called endothelium (Plate 5.4c). The cells are elongated in radial direction and perpendicular to the embryo sac (Plate 5.4 d), and are restricted around the middle portion of the nucellar plasmodium.

Plate- 5.4

a Mature embryo sac showing degenerated dyad (Dd) at thye micropylar end. See the egg apparatus (Eg) and chalazal polar nucleus.

b Fertilized egg called zygote (Zy) with degenerated dyad (Dd), degenerated synergid (Ds) and degenerated polar nucleus (Dpn).

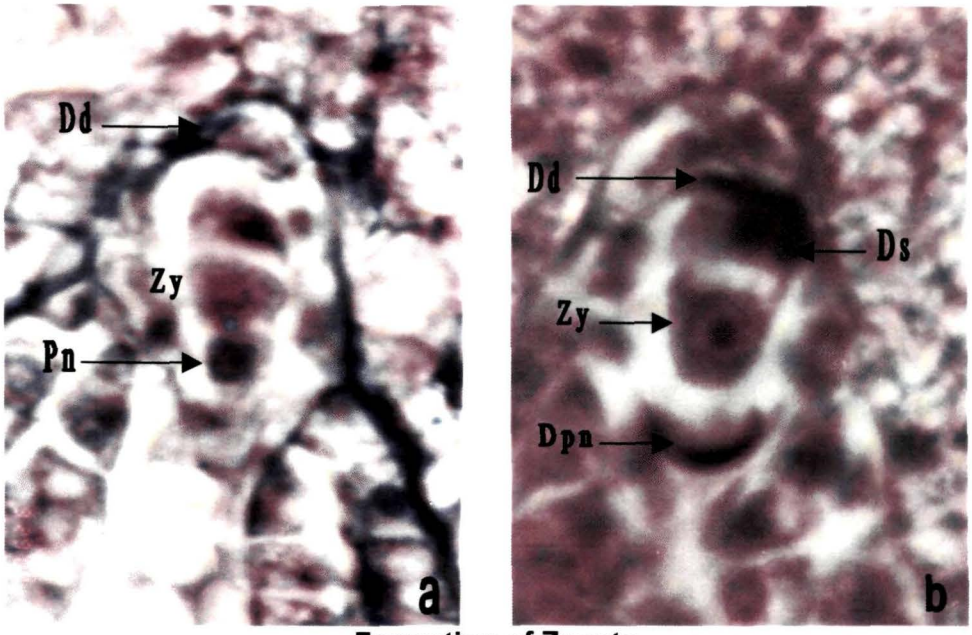
c SEM micrograph of ovule showing nucellar plasmodium (Np) enclosed within a cuticular sac (Cut). Note the radially stretched endothelium (En).

(a& b= x 1750)

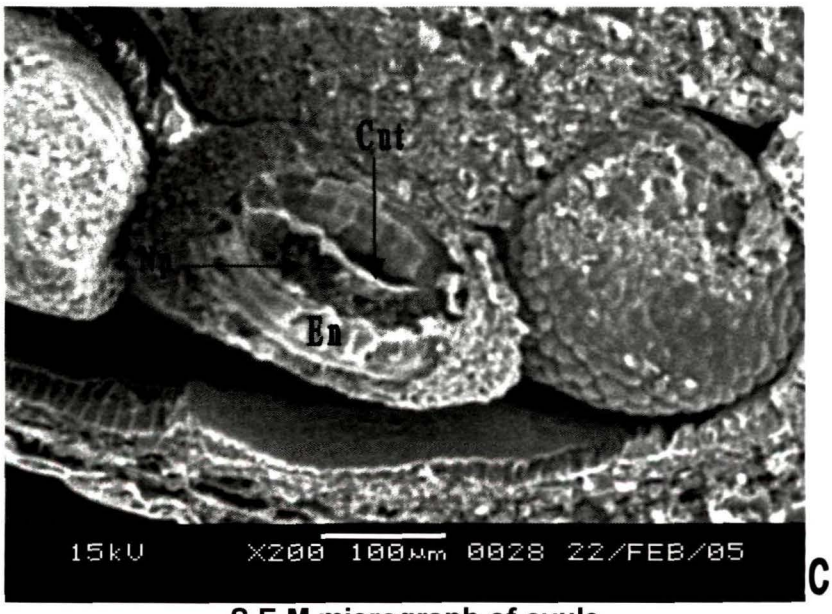
Plate- 5.4

***Polypleurum wallichii* (R. Brown ex Griff.) Warm.**

Embryogeny



Formation of Zygote



S.E.M micrograph of ovule

Plate- 5.4

d L .S of ovule showing two celled proembryo- basal cell (cb) and larger apical cell (ca) with dense cytoplasm. Degenerated dyad (Dd) and degenerated synergids (Ds) persists. Note the elongated cells of endothelium (En).

e Transverse division in both the cells result to form a 4- celled linear filamentous proembryo.

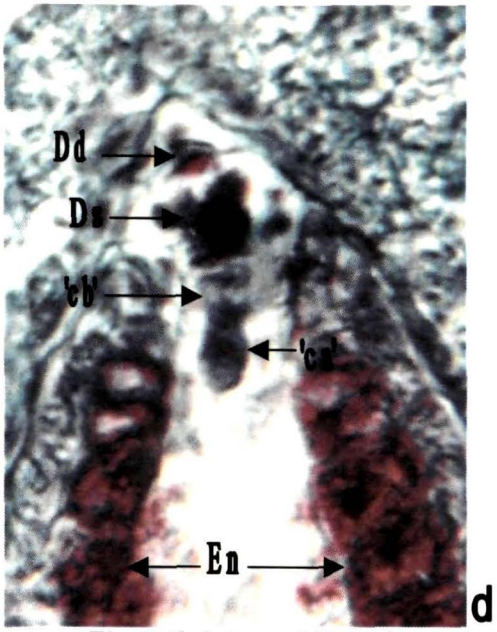
f Transverse division takes place in the derivatives of 'cb' whereas the most apically placed cell divides in a vertical plane.

g Another vertical division in 'cd' form a quadrant, arranged in two tiers. Note the distally placed cells contribute to the formation of suspensor haustoria.

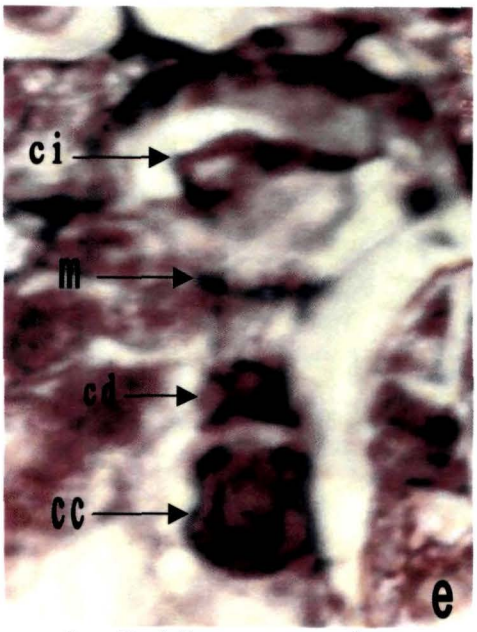
(d= x 950; e= x 1250; f= x 1600; g= 1500)

Plate- 5.4

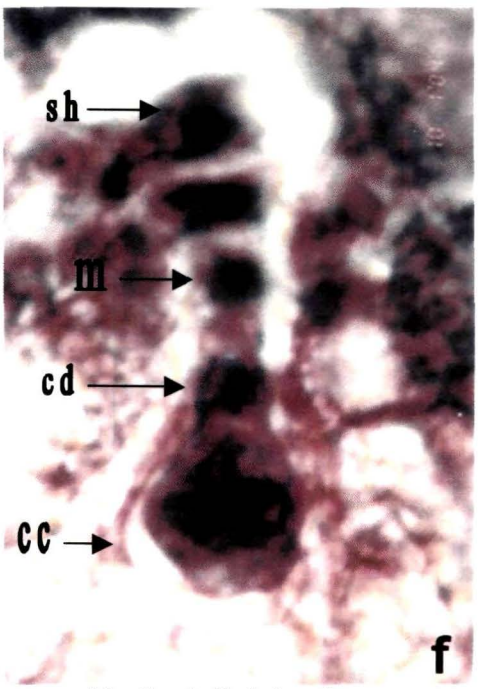
***Polypleurum wallichii* (R. Brown ex Griff.) Warm.**



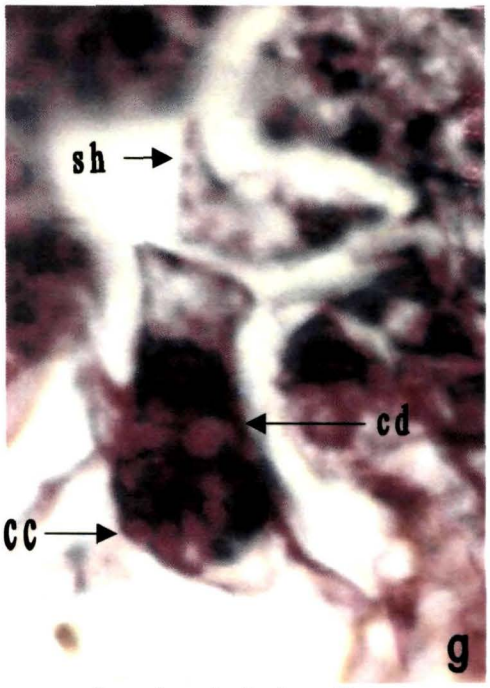
First division of Zygote



4 celled linear proembryo



Vertical division in ca



Quadrant in two tiers

Plate- 5.4

h The cells of quadrant divides to form 8- celled proembryo called octant; arranged in two tiers I and I'. The cotyledonary loci are differentiated in this stage of development.

i Various divisions in different plane result in the formation of globular proembryo (Gl).

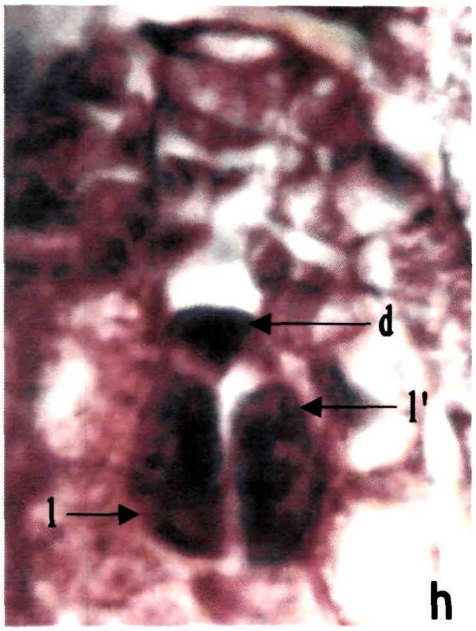
j Section of ovules in various plane showing the stages of pro embryo (pE) development.

k An enlarged view of single ovule with developing pro embryo.

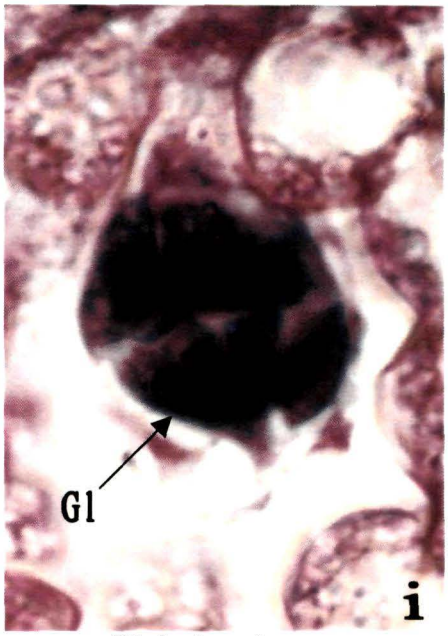
(h= x 1100; i= x 1350; j= x 20; k= x 50)

Plate- 5.4

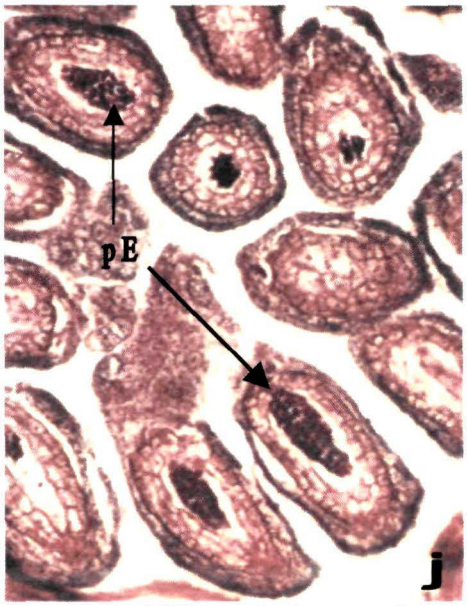
Polypleurum wallichii (R. Brown ex Griff.) Warm

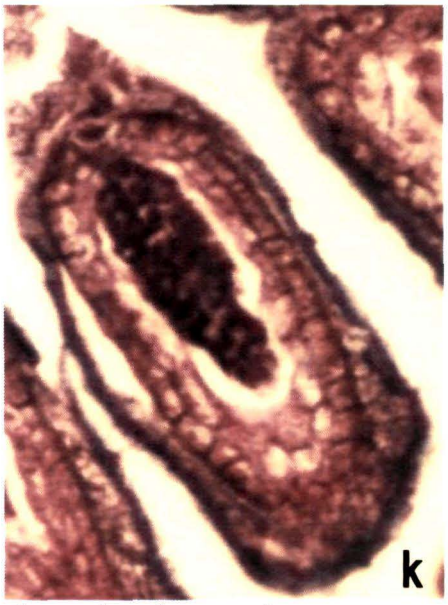


Octant proembryo



Globular stage





Different stages of pro embryo development

Plate- 5.4

l Dissected embryo possesses two cotyledons (Cot); suspensor haustoria (Sh) looks like thread.

m Mature embryo have two cotyledons (Cot). Shoot apex and note the aborted radicle (Ar) as brownish colour.

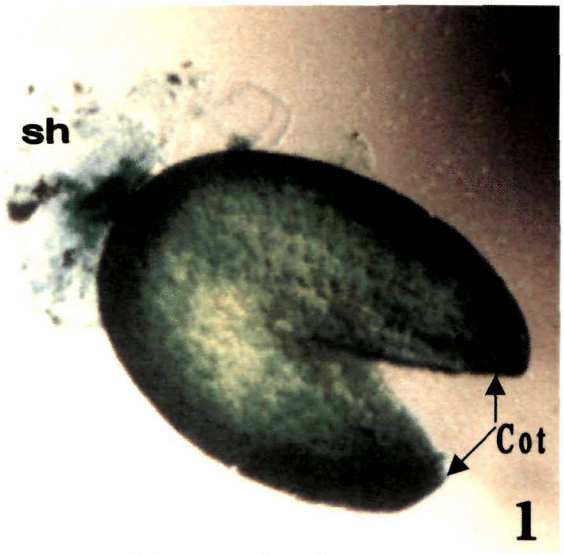
n Rhizoids (Rh) as hypocotyl outgrowth is the characteristic of Podostemaceae. Hypocotyl grows and emerges from seed coat (Sc).

o The cotyledons differentiates to form seedling (Ys) and emerges out of the seed coat (Sc).

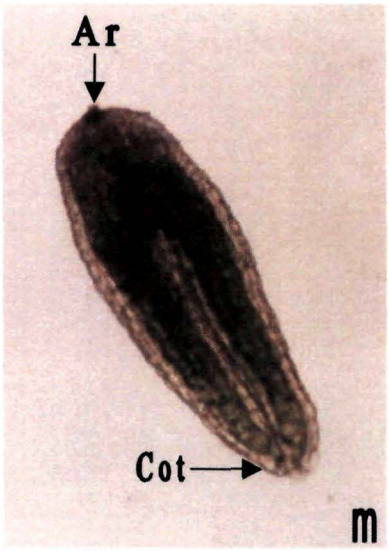
(l= x 60; m= x 70; n= x 40; o= x 30)

Plate- 5.4

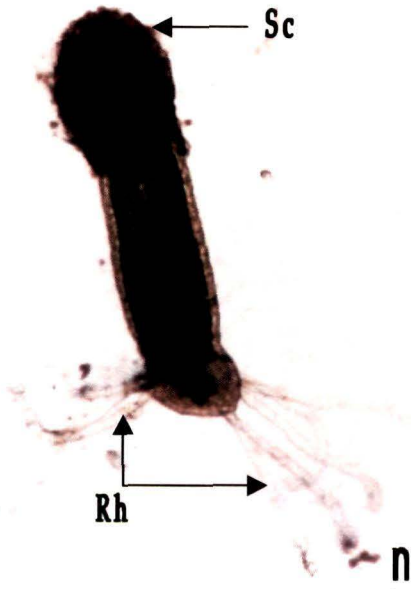
Polypleurum wallichii (R. Brown ex Griff.) Warm.

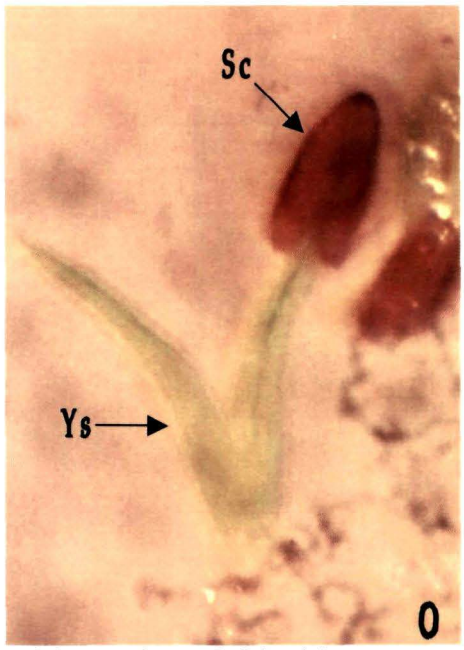


Dissected embryo



Mature embryo





Emergence of cotyledons and formation of rhizoid during Seed germination

CHAPTER - 6

Podostemum subulatus

Podostemum subulatus Gardner (In Calc. Journ. Nat. Hist. vii. 184, 1846; FBI. 5: 65, 1886; *Podostemum subulatus* var. *mavaeliae* Willis, var. *sholaii* Willis, *Zeylandium subulatus* (Gardner), C. cusset). *Podostemum subulatus,* Gardn. Tul. in Ann. Sc. Nat. Ser. 3, xi. 103, and Monogr. Podost. 135, t. 9, f. 4; Wedd. in DC. Prodr. xvii. 74; Wight Ic. t. 1918, f. 1; *Thwaites Enum*. 222. P. dendroides, *Thw. Mss.*) Willis in Ann. R. bot. Gdns Peradeniya I (3): 229, 1902; Engl. In Engl. & Prantl, Nat. Pflanzenfam. 18a: 63, 1930.)

6.1 Morphology and habitat

6.1.1 Habit

The plants are seasonal, submerged herbs in rapid mountain streams (Plate 6.1a). It is a minute flat, veined lobulate frond; buds on the edges of the lobes continuous with the veins. Thallus filiform, thread or ribbon like, elongate, branched, creeping and net veined; closely attached to rocks, about 3mm wide (Plate 6.1g). Secondary shoots ascending. Primary axis in the early stages gave rise to thallus, which is actually a plant that creeps on

the rock. Leaves long and slender, up to 30 mms long, very dense and obscuring the root when viewed from above (Plate 6.1 b). The long and subulate leaves, attached to rocks in tufts are the very characteristics of the species (Plate 6.1c). The dry branched thallus vestiges to the rocks at the end of the season (Plate 6.1i)

Flowers zygomorphic, naked, terminal with tubular or funnel shaped crest of about 3.0-3.5 mm long (Plate 6.1d). Borne in axils of leaves, several with long subulate leaves and no scaly bracts. Spathella elongate, funnel shaped, splitting irregularly at the tip (Plate 6.1g). Stamens 2, with transparent staminodes on each side at the base of ovary, 2-3 mm long; Anther lobes 0.9-1.2 mm long, 0.4-0.6 mm broad, pale yellow in colour (Plate 6.1e).

Flowering starts in late august and fruiting observed in early October; seeds dispersal at the middle of November. Ovary ellipsoid, 2-locular, style short with small papillae, green in colour, 4-5 mm long. Pedicel 2-3 mm long (Plate 6.1d). Stigmas 2, bifid 0.5-1.0 mm long, Fruit a capsule, unequally lobed, 8-ribbed (Plate 6.1f &h). Tips of capsule valves rounded, not wing-like. Valves persistent incurved. Seeds minute, many and oval (Plate 6.1j); testa membranous and transparent; tegment dark brown; length varies from 210µ-260µ and breadth 90µ-110µ (Plate 6.1k).

6.1.2 *Locality*

The specimen collection is made from the stream near Umtienger (Plate 6.1a), about 22 kms away from Shillong, East khasi Hills District, Meghalaya, India (91° 70'N – 25° 40'E).

6.1.3 Key characters for Identification

1. Cotyledons two.

   -Dicotyledons

2. Fruit a capsule. Plants attatched to rocks by disc like processes; appearing like algae or bryophytes.

   -Podostemaceae

3. Perianth of 2, rarely 3 scales. Stamen 1 or 2-3 with filaments connate.

   -Eupodostemaceae

4. Stigmas short, linear or ovate, entire, stamens 2. Flowers with a tubular or funnel shaped crest. Spathe tubular.

   -*Podostemum*

5. Thallus filiform, obscured by dense leaves when viewed from above. Ovary 8-ribbed.

   P. subulatus

Plate- 6.1

a River habitat in Umtienger locality. See the exposure of plants by receding of water level.

b Habit of plant with thread like filiform thallus (Th). Long subulate leaves (L) are the characteristic of the species.

c SEM of subulate leaves (L). New leaves sprout from the base of old leaves.

d Bisexual flower showing young ovary (O) with fertile stamens (Sm) on the biforked filament (F). See the staminode (St) at the base of the ovary.

e An enlarged view of stamen showing bilobed anthers (S) in a biforked filament (F).

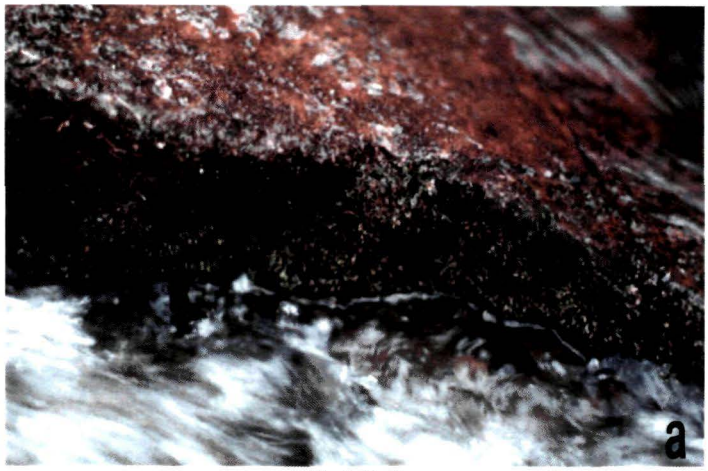
f SEM of a single mature ovary (O). Note the development longitudinal ridges.

(d= x 2; e= x 50)

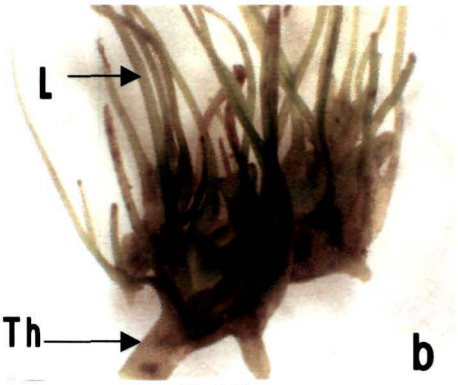
Plate- 6.1

***Podostemum subulatus* Gardn.**

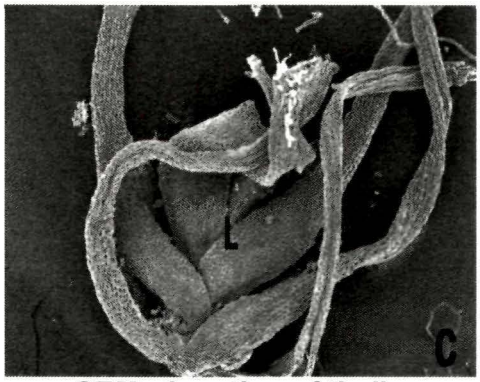
Morphology and Habitat



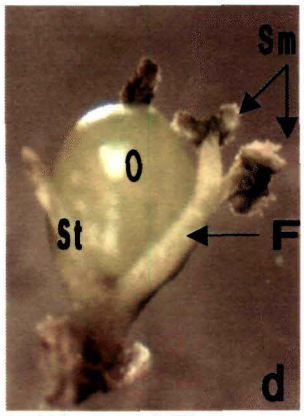
Habitat



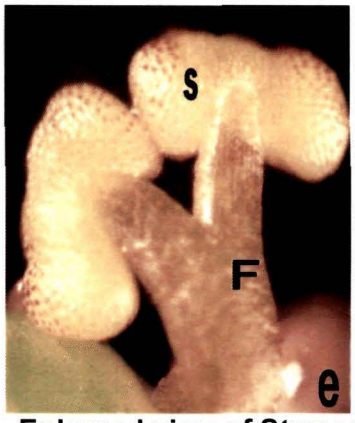
Habit



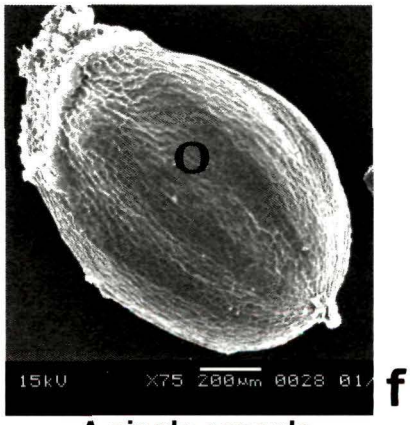
SEM of portion of thallus



Bisexual Flower



Enlarged view of Stamen



A single capsule

Plate- 6.1

g Ribbon like thallus (Th) with leaves (L). Note the buds (O) covered by bracts (Br) on the edges of the lobes continuous with the veins.

h Habitat with mature capsules on the dried thallus after the monsoon period on rocks.

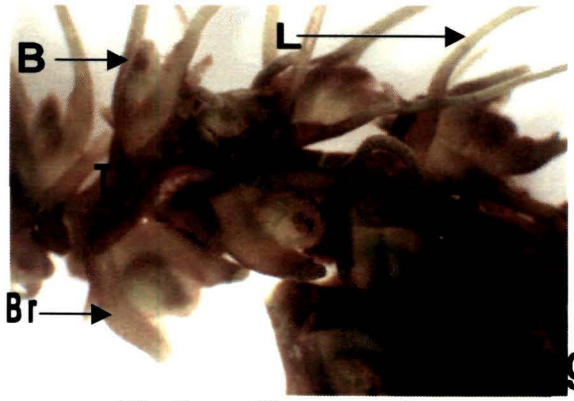
i At the end of the season, variously branched dry thalli visible on the rocks looking like lichens.

j& k SEM of seeds. Note the large, distinct ridges and grooves with the smooth micropylar region.

(g= x 2: h= x $^{1}/_{2}$)

Plate- 6.1

Podostemum subulatus Gardn.

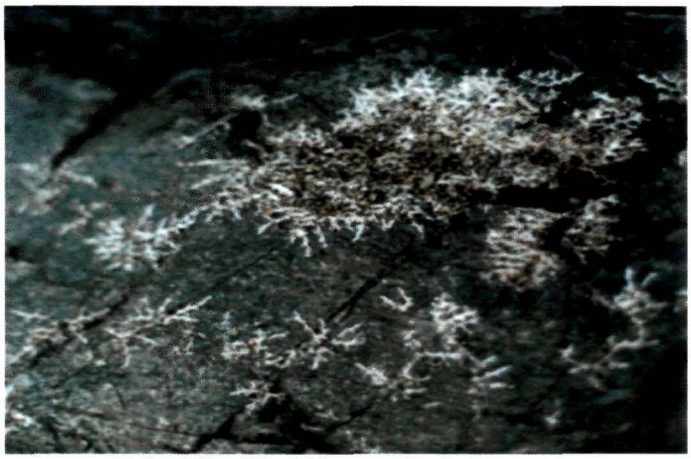


g

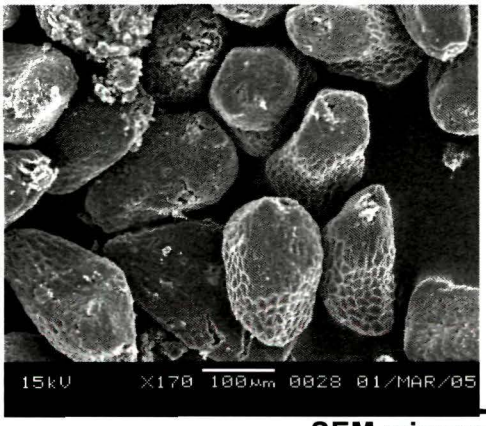
h

Thallus with young flowers

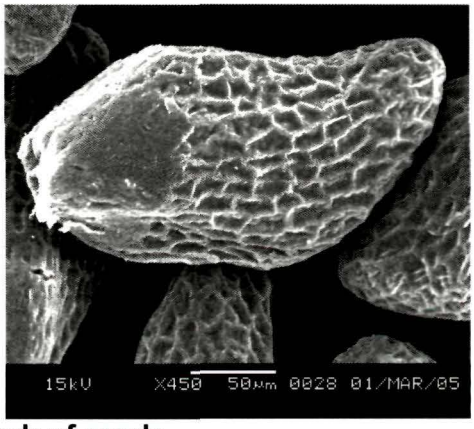
Fruits (Capsules)



Dried thalli at the end of a season



j



k

SEM micrograph of seeds

6.2 Anther Development and microsporogenesis

6.2.1 *Initiation of Microsporangium*

The stamens are bifid; each lobe contains tetrasporangiate anther lobes (Plate 6.2a & p). The two stamen primordia developed prior to gynoecium (Plate 6.2c). On initiation, the staminal primordium comprises a mass of undifferentiated meristematic cells and each primordium comprises of unilayered dermatogen that covers the multicellular hump-like tissues (Plate 6.2b).

The two primordia of staminode arise at the base of fertile stamen, enclosed by a perianth (spathe) (Plate 6.2 a). Ontogenetically the bifid stamens are two independent units; because of intercalary growth at the base of andropodium, the filament grows into a biforked or bifid structure (Plate 6.2c & d) and each fork produce a tetrasporangiate anther in each fork. Each stamen primordium after reaching 10- 12 µm in length, the vasculature is differentiated in an acropetal direction. The four anther primordia in each lobe are differentiated on the adaxial face, so that, all the four lobes of each anther facing towards the stigma (Plate 6.2 e).

6.2.2 *Archesporial cell and Anther wall formation*

A plate of 4-6 hypodermal cells is differentiated into an archesporial initials in each anther lobe (Plate 6.2 b). Archesporial cells are radially elongated, densely cytoplasmic with a large, more prominent nucleus (Plate 6.2 f). The entire archesporial initials divide periclinally, to form two layers. The inner layer becomes primary sporogenous cell and the latter one primary parietal cells. The primary sporogenous cell divide and re-divides to form sporogenous tissue.

The primary parietal cells undergo only one periclinal division to form two-layered secondary parietal layer, which directly rebuild into endothecium and tapetum respectively, the middle layers are absent (Plate 6.2 g). Therefore, the ontogenic development of anther wall layer belongs to reduced type. The endothecial layer as well as parenchymatous cells adjacent to the connective tissue also acquires fibrous annular thickenings that develop at the time of meiosis-II in the meiocytes.

6.2.3 *Tapetum*

Tapetum is secretory, unilayered in structure; occasionally a few cells becomes bi-layer towards the connective tissue because of oblique division in these tapetal cells, enclosing the sporogenous tissues (Plate 6.2 f). The cells are rectangular, both uni and bi-nucleate condition cells are seen (Plate 6.2 l). The cells

are densely cytoplasmic with lots of starch and protein contents. The entire tapetum is derived from inner layer of the secondary parietal layer (Plate 6.2g). The tapetum releases granular substances that are similar to that of sporopollenin deposited on the inner tangential wall, lining towards the anther locule. The tapetal cells remain until the liberation of pollen grain in pairs (Plate 6.2 l& m).

6.2.4 *Meiosis and Cytokinesis*

Prior to meiosis, the microspore mother cells becomes rounded and separated out (Plate 6.2 g). The microspore mother cells are enclosed by a distinct callose wall that gets dissolved after the completion of meiosis-II and before the onset of pollen wall deposition. Meiosis is uniform in all the microspore mother cells in each theca, but not in adjacent anther locules (Plate 6.2g& l). Meiosis is of successive type. In meiosis- I, the chromosomes are arranged in the equatorial plane (Plate 6.2h& l); and a dyad is formed at the end of meiosis- I (Plate 6.2 j). After the first meiotic division, a distinct cell plate is formed and the nuclear spindle disappeared (Plate 6.2 k). As soon as the first meiotic division is over, the nuclei of daughter cells immediately undergo second meiotic division (homeotypic division) without any resting period in which the spindles of metaphase-II are either parallel or right angles to each other (Plate 6.2 l). A tetrad resulted in second

meiotic division (Plate 6.2 m), which then get separated and the pollen grain are dispersed in pairs (Plate 6.2 q).

During and after meiosis-II, the pro-orbicules (Ubisch bodies) are released from tapetum and deposited on the pollen exine (Plate- 6.2n& o). The cytoplasm does not increase rapidly enough to fill the entire lumen of the microspore wall during the initial stage of development. Because of vacoulation, the microspores enlarge to their maximum size; the cytoplasm forms only a thin layer around nucleus with radiating strands (Plate 6.2 q). Subsequently, the cytoplasm increases quickly and the nucleus shifts its position to the distal pole where it divides to form the vegetative and generative cells. Because of the callose wall (Plate 6.2 m), pollen grains fluoresce brightly in fluorescence microscope. Pollen germination on the stigmatic surface in natural condition has also been observed (Plate 6.2 s).

6.2.5 *Pollen grains*

Pollen grains in *Podostemum* also occur in pairs (Plate 6.2 n). Dyads are 19-25µm long and 10-12µm broad. The shared wall between the two grains averages 8.7µm wide. Individual grains are spherical (Plate 6.2 q) and tri- aperturate. Apertures are best characterized as colpi; however they have weakly defined margins and looks like an oval shaped furrows with broadly rounded ends. The pollen surface is also microechinate (Plate 6.2 o); however

the sculptural elements on the non-apertural wall do not have broadened basal pads. By comparison, the microechinate on the apertural surface are more distinct as well as more pointed. Exine 1µm thick. The distribution of the processes follows a definite pattern.

6.2.6 Pollen viability

The pollen viability of *Podostemum subulatus* is examined by using Fluorochromatic Reaction (FCR) test. The viable pollen fluoresces brightly and non-viable one didn't fluoresce (Plate 6.2 r). It is observed that only 36.17 % of pollen grains were viable, which is comparatively low in contrast to the other two species studied. ()

Table – 6.2.6: Pollen viability test by using FCR Test

Name of Plants	Total no. of pollens		No. of viable pollens		Percentage of viability
Podotemum subulatus Gardn	R_1	58	R_1	21	36.17± 3.21
	R_2	101	R_2	52	
	R_3	196	R_3	74	
	R_4	136	R_4	46	
	R_5	131	R_5	32	
	Mean	124.4	**Mean**	45	

± : Standard deviation (SD)

R_X: Number of replicates

Plate- 6.2

a L. S of young flower bud showing two stamen primordia (Sp) with staminodes (St) on either side of common axis. The perianth (Pr) encloses the young flower.

b An enlarged view of anther primordia showing rapid cell division below the epidermal layer (E).

c L. S of young flower showing an elongation of anther primordium (Sp). See the meristematic growth of the andropodium (Ap) and differentiation of vasculasture.

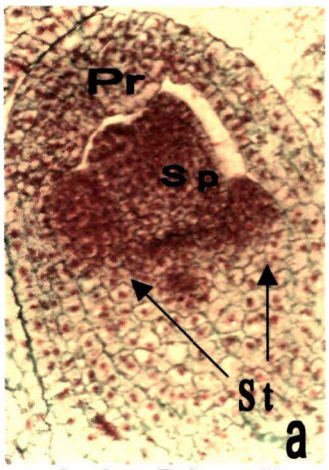
d L. S of flower showing biforked filament (F) as a result of intercalary meristematic growth in the andropodium with anthers (An) within the perianth (Pr) cover.

(a= x 100; b= x 300; c= x 90; d= x 30)

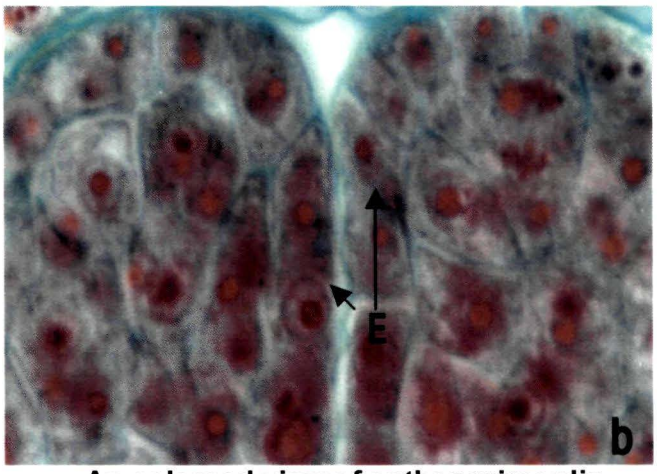
Plate- 6.2

***Podostemum subulatus* Gardn.**

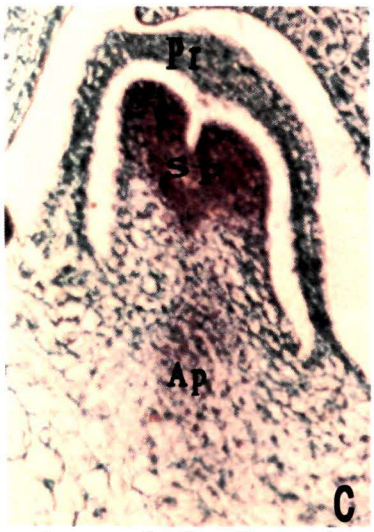
Anther Development and Microsporogenesis



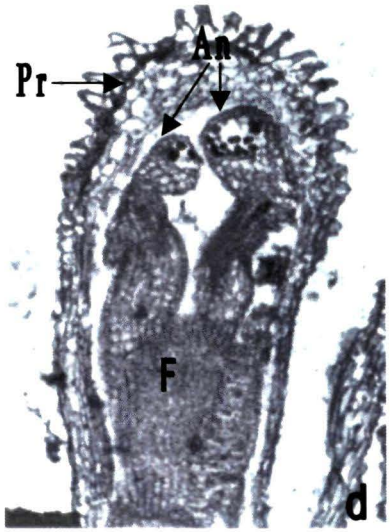
Anther Primordia



An enlarged view of anther primordia



Development of anther primodia



Bifid stamen on andropodium

Plate- 6.2

e L. S of young flower showing gynoecium (G) and two tetralocular anthers (An).

f An enlarged view of single anther lobe showing sporogenous tissue (Sc) enclosed by tapetum (Tp) and epidermis (E).

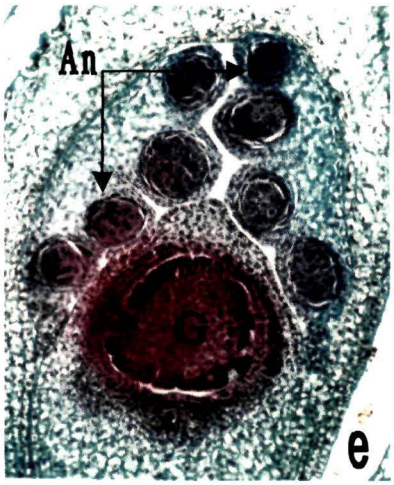
g A single anther lobe showing microspore mother cells (mmc). See the distinct binucleate condition of tapetum (Tp) , endodermis (Em) with the outermost epidermis (E).

h& i Stages of meiosis- I in the microspore mother cells. Note the chromosome and nuclear spindles (Ns) in the equatorial plate.

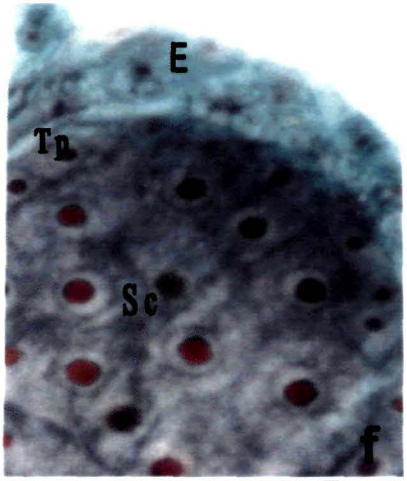
(e= x 120; f= x 300; f= x 550; h& i= 1300)

Plate- 6.2

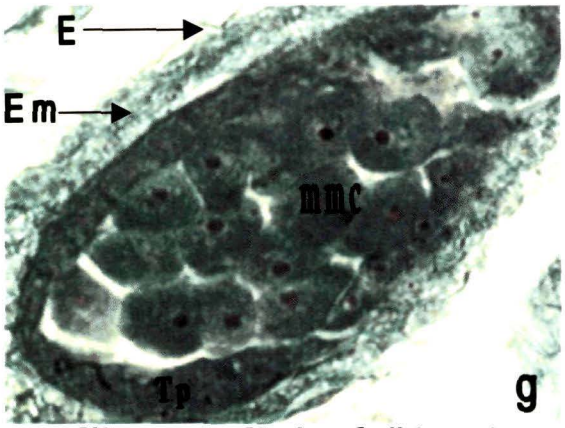
***Podostemum subulatus* Gardn.**



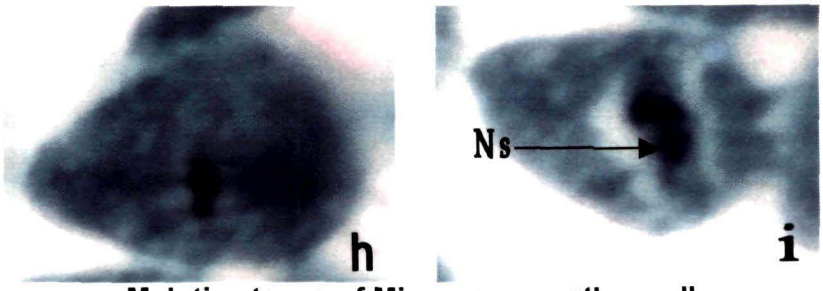
L. S of young flower



Sporogenous tissue with Tapetum



Microspore Mother Cell (mmc)



Meiotic stages of Microspore mother cell

Plate- 6.2

j& k Meiosis result to dyad (D) formation accompanied by cell plate formation.

l Dyad cells undergo meiosis- II to form tetrads. The tapetum (Tp) is binucleate.

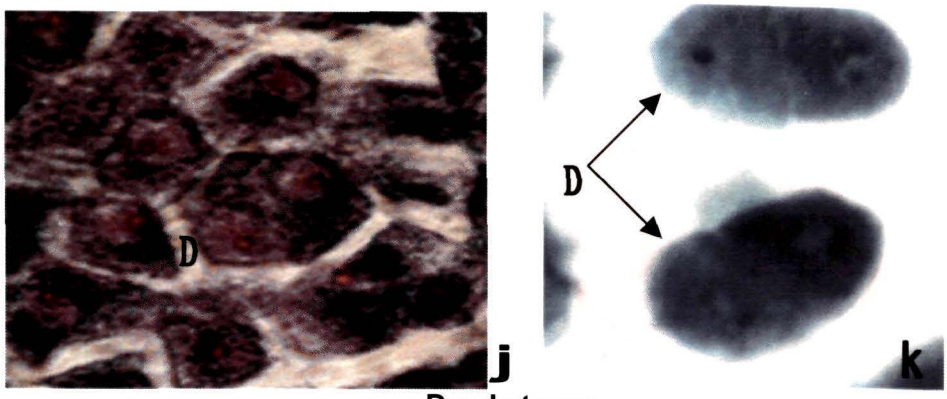
m A thin callose wall holds the tetrad together.

n& o SEM of a dyad pollen grains. Microechinate ornamentation is distinct on the pollen walls.

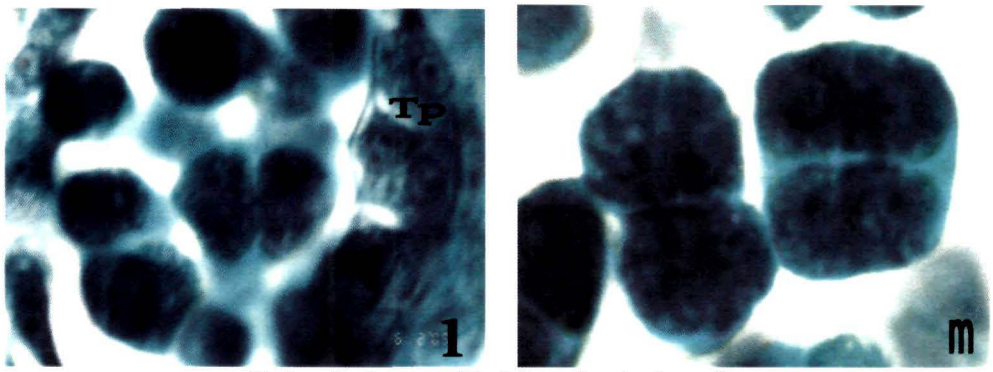
(j= x 400; k= x 1250; l= x 375; m= x 1800)

Plate- 6.2

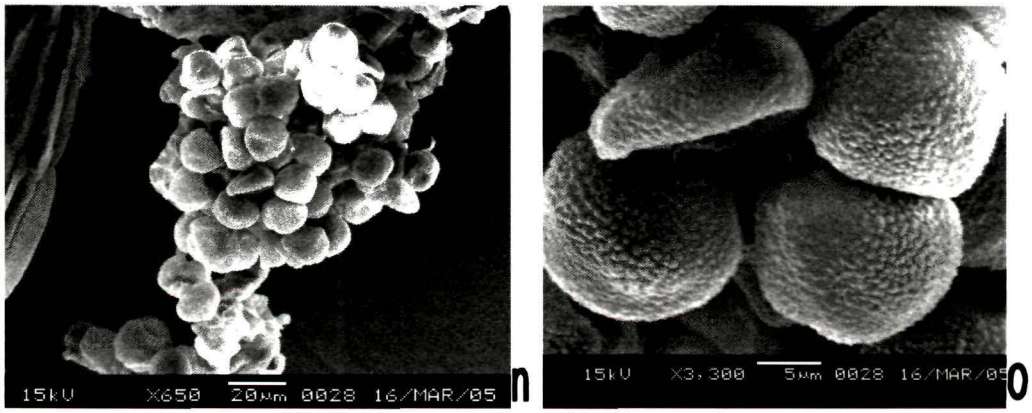
Podostemum subulatus Gardn.



Dyad stages



Tetrad stage with binucleate tapetum



SEM micrographs of pollen grains

Plate- 6.2

p Transverse section of stamen showing tetra locular anther (An) with pollen grains.

q Mature pollen grain. Note the pollen grains are dispersed in pair.

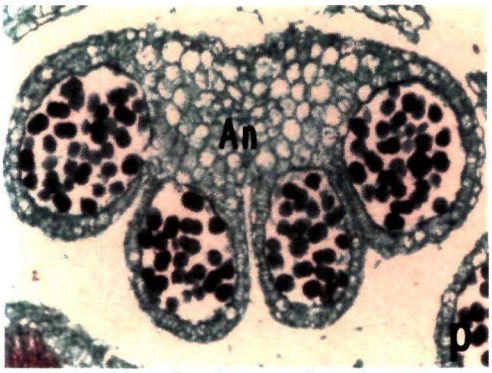
r Pollen viability test with FCR test. Only the viable pollens give fluorescence.

s Stigmatic surface (Sm) under fluorescence microscope showing pollen germinations in natural condition.

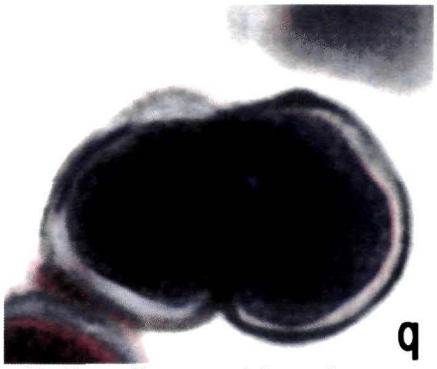
(p= x 225; q= x 2250; r= x 200; s= x 350)

Plate- 6.2

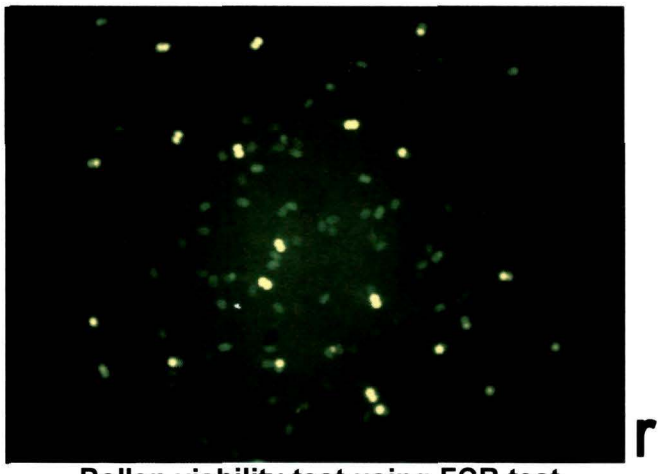
***Podostemum subulatus* Gard.**



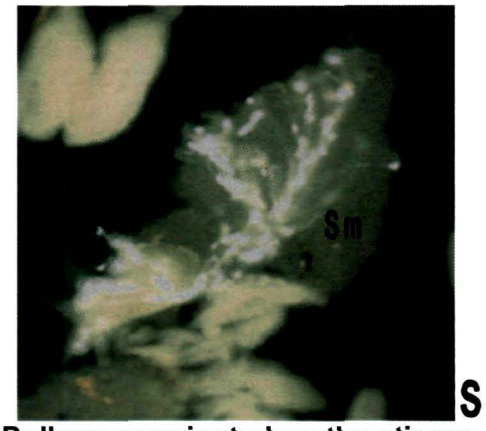
Pollens in the anther locule



Pollen dispersal in pairs



Pollen viability test using FCR test



Pollens germinated on the stigma

6.3 Megasporogenesis and Embryo sac development

6.3.1 *Megasporangium*

Ovule numerous on axile placentation (Plate 6.3 a& b). The ovular primordia arise as small protuberance consisting of seven cells in cross section i.e. central single row of cells covered by six cells, on the placenta (Plate 6.3 d). As soon as the outer integument differentiates on the ovule primordium, the tenuinucellus is demarcated (Plate 6.3 e). A curvature of 180° is then attained in the ovule because of unequal growth rate in the chalazal portion so as to reach anatropous condition (Plate 6.3 c).

6.3.2 *Megasporogenesis*

In the tenuinucellate ovule, a single hypodermal cell becomes archesporial initial, which is distinguished by its large size, dense cytoplasm, with distinct nucleus and nucleolus (Plate 6.3 e). The archesporial initial directly functions as megaspore mother cell, which become elongated in apical to basal axis; the protoplast is separated from the nucellar epidermis (Plate 6.3 f).

6.3.3 *Embryo sac development (Megagametogenesis)*

In the first division of meiosis, the nucleus moves towards the micropylar side where it undergoes heterotypic meiotic division to form two cells (Plate 6.3 g). Out of the two cells, the micropylar

one degenerate to form the apical cap, whereas the chalazal cell undergoes 2nd meiotic division to result into two-celled condition. Here also, similar to the previous division, the chalazal cell degenerate and disappear (Plate 6.3 h). The remaining centrally located cell is functional for the formation of embryo sac, called megaspore; conforming to monosporic type. The megaspore then undergoes two cycles of mitotic divisions, resulting into 4-celled embryo sac structure, which organizes with two synergids located at the micropylar end, a single cell at chalazal end designated as polar cell or nucleus (Plate 6.3 i). A large egg cell located in the central median plane. The egg cell has comparatively larger nucleus and dense cytoplasm than the rest of the cells of embryo sac.

Plate- 6.3

a L. S of young flower showing gynoecium (G), stigma (Sm) and stamen (S).

b T.S of young ovary showing ovule primordium (Op) on a thick placenta (Pl).

c T. S of young flower showing ovule initiation. Note the development of ovule primordium (Op) and exile placentation (Pl).

d Single ovule primordium showing nucellar cells (Nu).

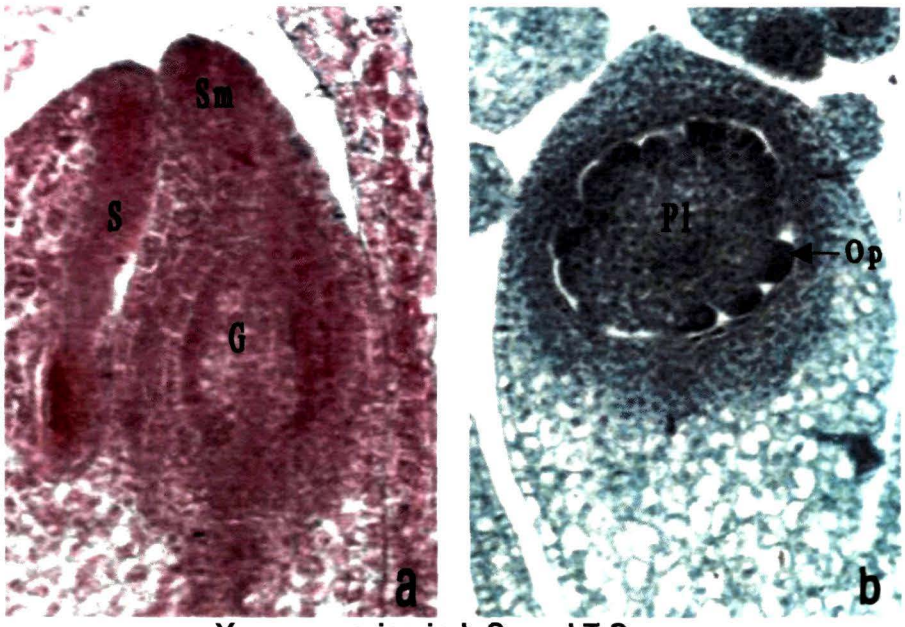
e Single ovule showing archesporial initial (Ai) and vertical row of nucellus (Nu). Note the outer integument (Oi) and initiation of inner integument (Ii) and ovary wall (Ow).

(a= x 400; b= x 200; c= x 160; d= x 1600; e= x 1800)

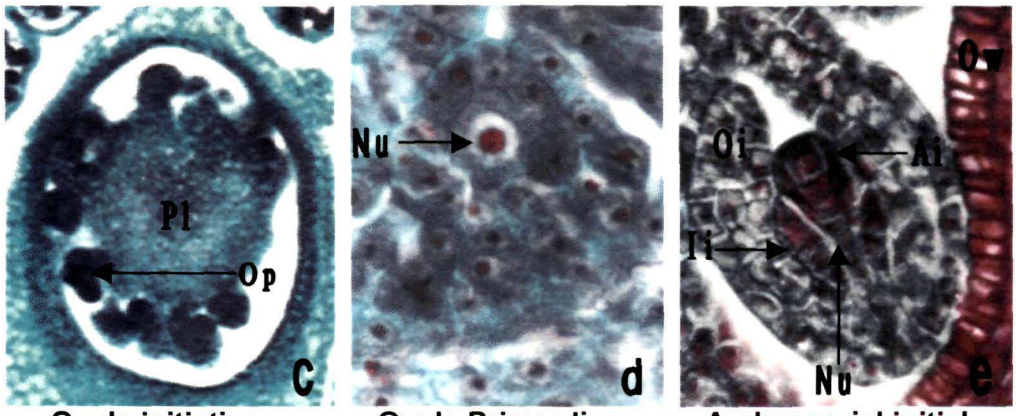
Plate- 6.3

Podostemum subulatus Gard.

Megasporogenesis and Embryo sac formation



Young ovaries in L.S. and T.S.



Ovule initiation | Ovule Primordium | Archesporial initial

Plate- 6.3

f Tenuinucellate ovule showing megaspore mother cell (MMC) with large central nucleus and nucleolus. See the nucellar cells (Nu) and development of inner integument (Ii).

g Meiosis in the MMC result in the formation of dyad (D). Note the coenocytic condition of the nucellar plasmodium (Np).

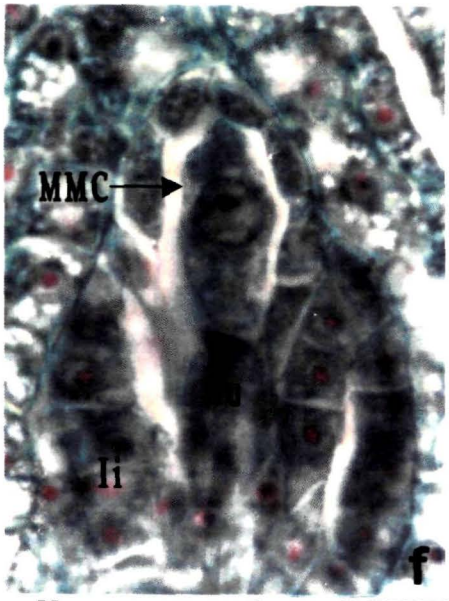
h L. S of ovule showing uninucleate functional megaspore (Fm) with the degenerating chalazal dyad nucleus. See the degenerated dyad (Dd) at the micropylar end. Also note the coenocytic nucellar plasmodium (Np).

i Four nucleate embryosac formed after two mitotic divisions in the functionalmegaspore. See the synergids (Ss) and egg cell (Ec) to form egg apparatus and the chalazal polar nucleus (Pn). The degenerated dyad (Dd) persist at the micropylar end.

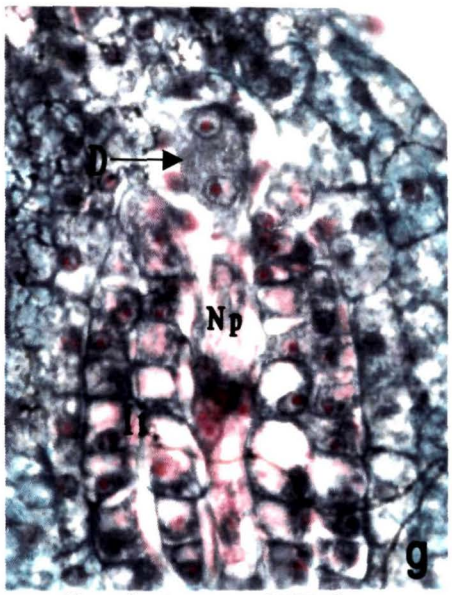
(f= x 2500; g= x 1500; h= x 2000; i= x 3000)

Plate- 6.3

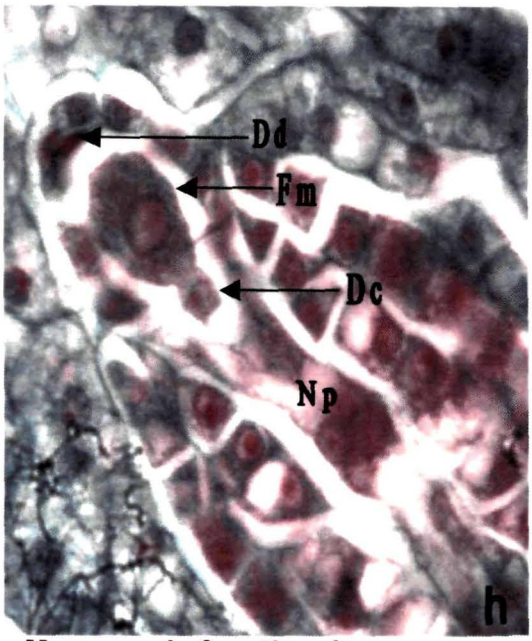
Podostemum subulatus Gard.



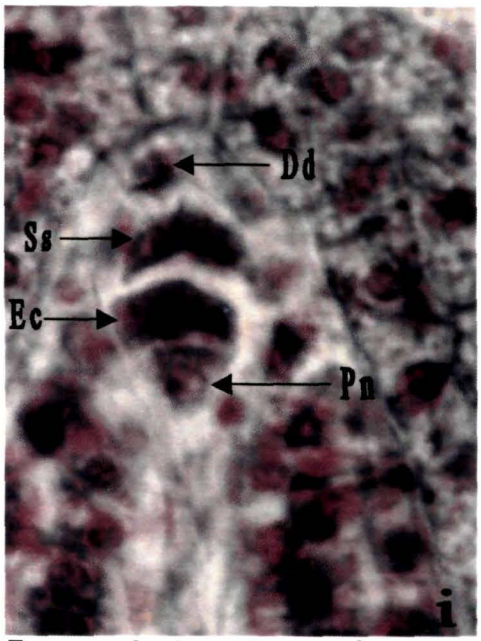
Megaspore mother cell (MMC)



Dyad stages of MMC



Monosporic functional megaspore



Four nucleate mature embryo sac

6.4 Embryogeny

6.4.1 *Zygote*

Fertilization is porogamous; the pollen tube enters into one of the synergids. Out of the two male gametes or sperms, only one takes part in the fusion process with the egg cell (Plate 6.4 a). The fertilized egg called zygote has a large nucleus with dense cytoplasm (Plate 6.4 b); because of its increasing volume, enlarges towards the chalazal pole. The degenerated polar nucleus can be noticed during the enlargement of the zygote (Plate 6.4 c). The entire protoplast of the zygote is encircled by a thin cellulosic cell wall. The disintegrated synergids and the dyad cell persist as dark structures. The zygote occupies a major portion of the embryo sac (Plate 6.4 d).

6.4.2 Embryogenesis

In the first division of zygote is in transverse plane, thus giving rise to a superposed arrangement of two daughter cells (Plate 6.4 I). Of these, the cell that is nearest to the micropyle is the basal cell (Conventionally designated as 'cb') and the one that is away from the micropyle is the terminal call "Conventionally designated as 'ca'" (Plate 6.4 j) . The basal cell divides to form 'm' and 'ci'; of which 'ci' is located more basally and the 'm' is placed in between ca and ci. (i.e.- an intermediate position) to form a 4-

celled proembryo (Plate 6.4 l&m). The apical cell divides longitudinally so that a T-shaped tetrad is formed in which the daughter cells are adjoined in a single tier (Plate 6.4 k). The derivatives of basal cell contribute mostly to the formation of suspensor haustorium (Plate 6.4 n& o). The adjacently placed cells divide by a vertical wall at right angle to the previous partition, thus giving rise to a four-celled structure in two tiers (Plate 6.4 p& q). These four cells undergo the next division in a transverse plane, thereby producing the octant proembryo (Plate 6.4 r). The component cells of octant stage became organized in two superposed tiers (I and I^{I}) of four cells each (Plate 6.4 s& t).

The octant stage is followed by numerous cell divisions in various planes causing the pro embryo to assume a globular configuration (Plate 6.4 u& w). The cotyledonary loci and epiphyseal locus are differentiated from the globular proembryo (Plate 6.4 u& v). The proembryo undergoes a series of transformations to attain full differentiation (Plate 6.4 x,y,z& a'). A mature embryo consists of two cotyledons with wavy thread like suspensor haustoria. The embryo is devoid of plumule and radicular portion (Plate 6.4 b').

6.4.3 *Suspensor Haustorium*

The growth and development of suspensor is much expedious to the differentiation of embryo (Plate 6.4 m& n). When

the pro embryo reaches the octant stage, the differentiation of suspensor is almost completed.

The distal cell of the suspensor at the micropylar pole functions as the haustorium. The cell is comparatively large with several haustorial branches. The two nuclei in the haustorial cell are much larger than the rest of the cells, which indicate that these nuclei undergo endo-reduplication of DNA contents. The uppermost cell of the suspensor (n') divides vertically to form two cells lying side by side, which have been renewed into suspensor haustoria just below the micropyle (Plate 6.4 o). The nuclei of haustorial cells became hypertrophied and the branches strike in between the integuments, chalaza and micropyle to draw the nutrition (Plate 6.4 b').

6.4.4 *Nucellar Plasmodium*

It begins to develop at the time the megaspore mother cell undergoes meiosis-I. As soon as the MMC has started to divide, the nucellar cells stretched in longitudinal axis; the cell walls become thin and dissolve. So that the entire dissolution products such as cytoplasm, nuclei and dissolved materials form coenocytic plasmodium, termed as nucellar plasmodium (Plate 6.4e). The nucellar plasmodium is fully formed much before the complete organization of the embryo sac, which is delimited by a thin cuticular wall (Plate 6.4 h).

6.4.5 *Endothelium*

The inner integument consists of two layers of cells; the inner layer of inner integument, adjoining to nucellar plasmodium is rebuilt into integumentary tapetum called endothelium (Plate 6.4 f). The cells are radially elongated in perpendicular to the embryo sac (Plate 6.4 g). Endothelium is restricted around the middle portion of the nucellar plasmodium (Plate 6.4 h).

Plate- 6.4

a Mature embryo sac showing degenerated dyad (Dd), two synergids (Ss), egg cell (Ec) and polar nucleus (Pn). Note the degenerated chalazal dyad (Dc) persist as a red crescent structure.

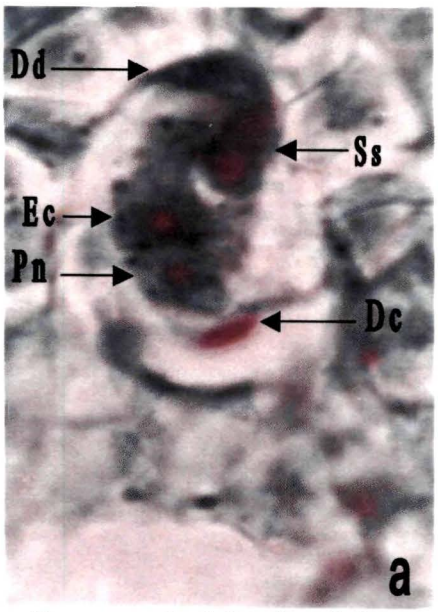
b- d L. S of ovary showing fertilized egg or zygote (Zy) in different views. See the degenerated dyad (Dd), degenerated synergids (Ds) and degenerated polar nucleus (Dpn). Note the distinct polarity of the zygote where the nucleus and other organelles are confined to the chalazal pole.

(a-c= x 3500; d= x 3200)

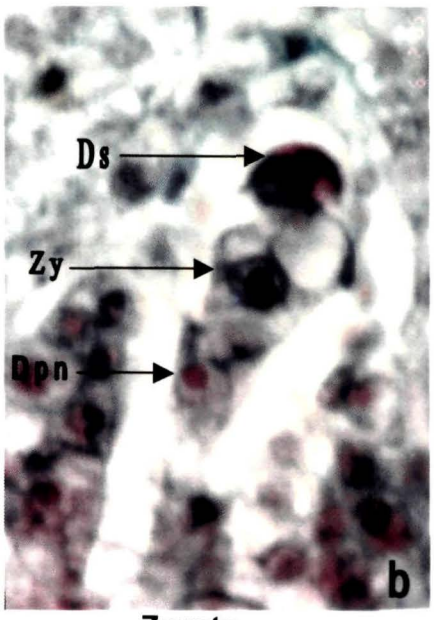
Plate- 6.4

Podostemum subulatus Gard.

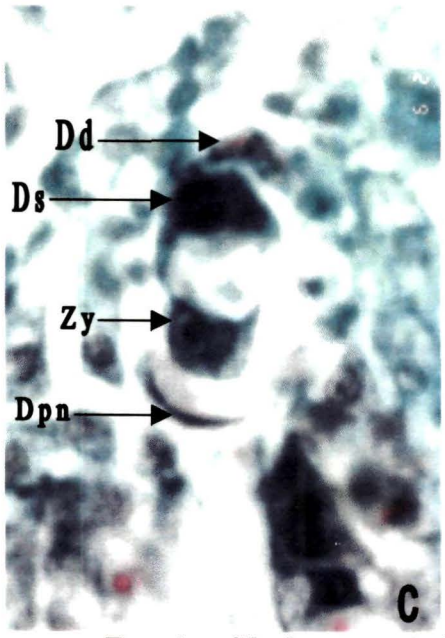
Embryogeny

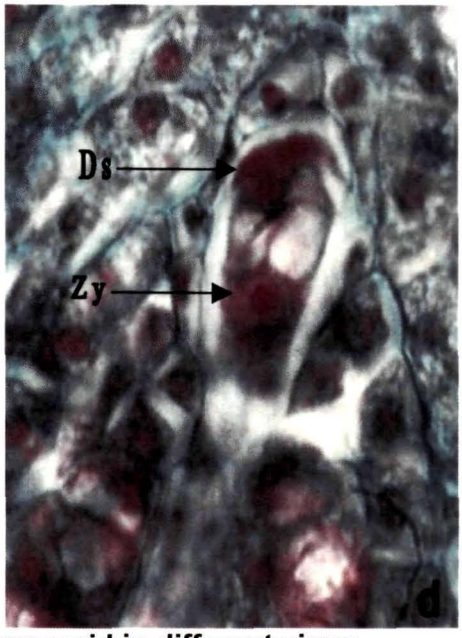


Degenerated Chalazal dyad



Zygote





Zygote with degenerated synergid in different views

Plate- 6.4

e L. S of ovary showing multinucleate condition of sac-like structure called nucellar plasmodium (Np) inside the sac like structure.

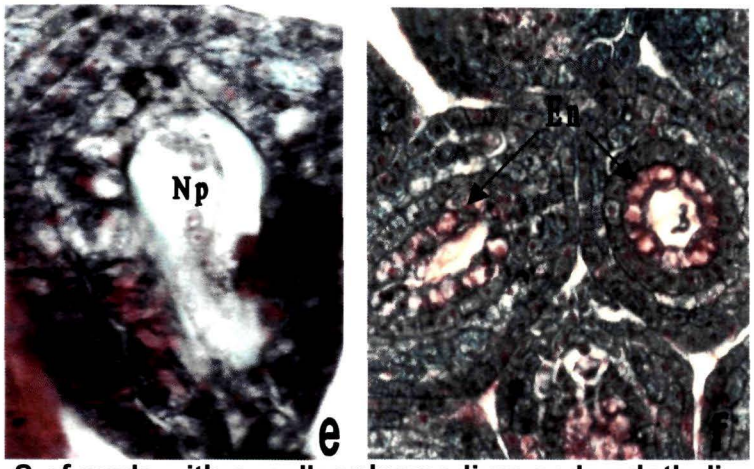
f& g Section of ovules in both T.S and L.S showing endothelium (En) formed from inner layer of inner integument and radial stretching of endothelial cells.

h SEM of ovary showing nucellar plasmodium (Np) enclosed within cuticle (Cut) and endothelium (En).

(e= x 850; f= x 275; g= x 220)

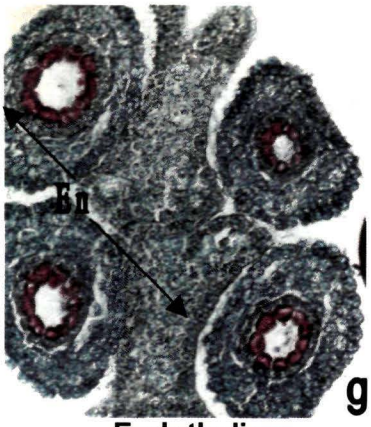
Plate- 6.4

***Podostemum subulatus* Gard.**



e f

L.S of ovule with nucellar plasmodium and endothelium



g

Endothelium

h

SEM micrograph of ovule

Plate- 6.4

i Two celled pro embryo. The apical cell (ca) have large nucleus and dense cytoplasm; larger in size compared to basal cell (cb).

j An enlarged view of two celled pro embryo. See the small size of distally placed basal cell (cb) and a larger apical cell (ca). Note the fully developed endothelium (En).

k Division of 'cb' precedes the 'ca' at transverse plane to form m, n and n'.

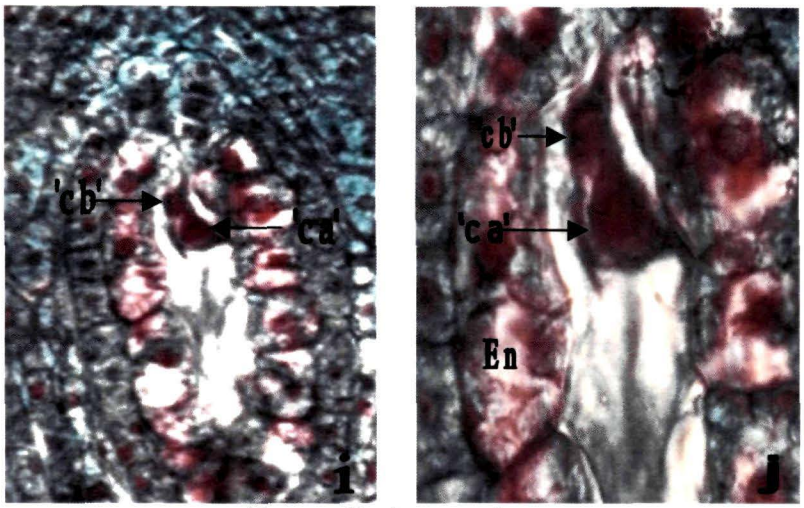
l Vertical division in apical cell 'ca' result in tetrad pro embryo.

m- 0 Different stages of cell division in the basal cell which contribute to the formation of suspensor and its haustorium (Sh).

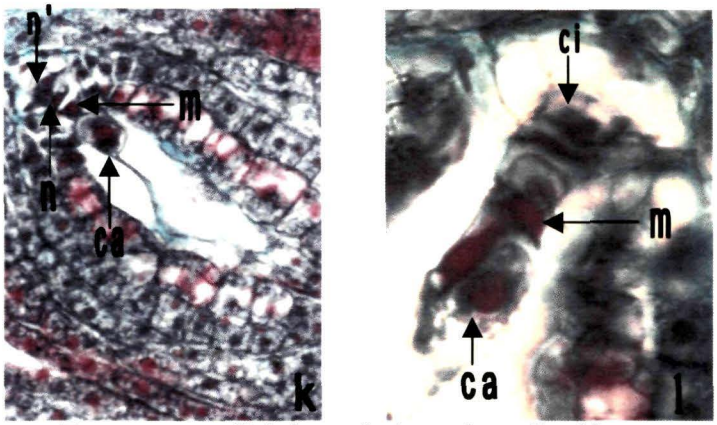
(i= x 1650; j= x 2200; k= x 900; l= x 2300; m-o= x 2300)

Plate- 6.4

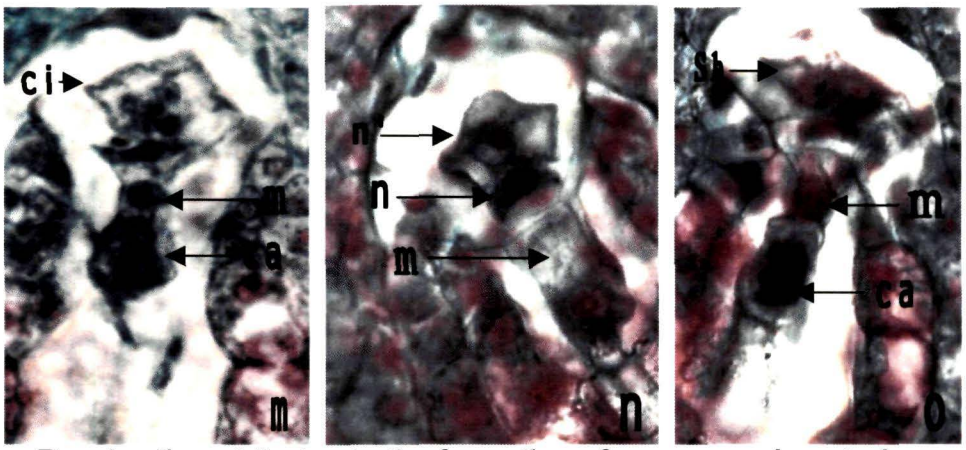
Podostemum subulatus Gard.



Two celled proembryo



Transverse division of cb and vertical in ca



Basal cell contributes to the formation of suspensor haustorium

Plate- 6.4

p& q Vertical division in apical cell (ca) result in tetrad pro embryo.

r Another division with cell plate formation produce 8- celled pro embryo called octant.

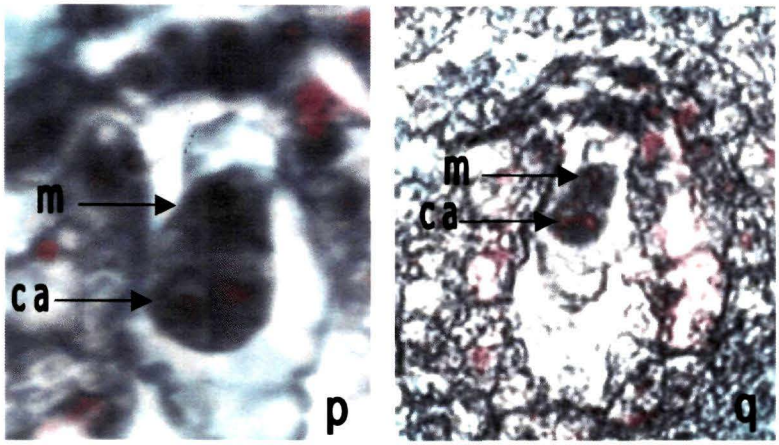
s& t The quadrant divides in a transverse plane result into a 16-nucleate stage. Cotyledonary loci are differentiated.

u- w Section of ovary in longitudinal and planes transverse. Numerous cell divisions in various planes result globular shaped proembryo (Gl). The proembryo passes through a phase before the cotyledons and epicotyls become outwardly evident at a specified loci.

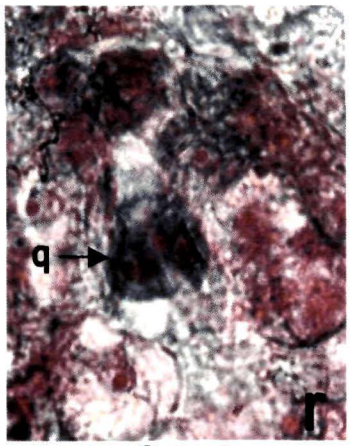
(p= x 2500; q= x 1100; r= x 2200; s= x 2500; t= x 2600; u- w= x 2600)

Plate- 6.4

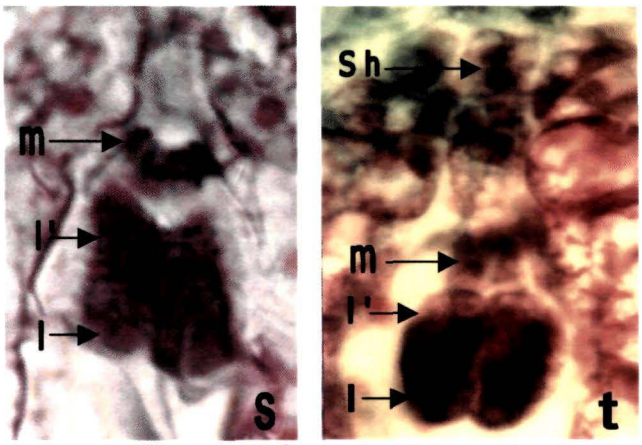
***Podostemum subulatus* Gard.**



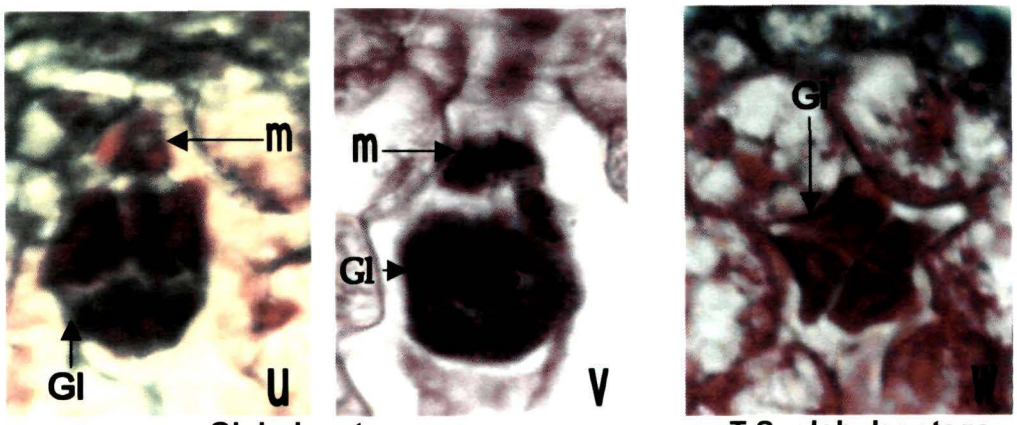
Tetrad proembryo



Octant



16-nucleate stage



Globular stages

T.S. globular stage

Plate- 6.4

x& y Elongated proembryo under low and high magnification. The pro embryo (pE) is elongated to attain a torpedo stage.

z T. S of ovule showing mature embryo with two cotyledons.

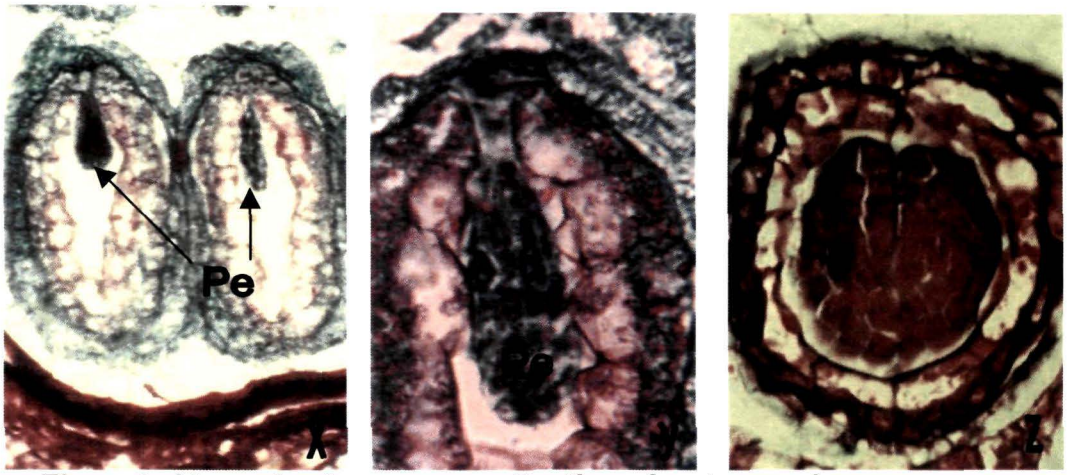
a' Mature embryo showing distinct cotyledonary loci (Cot).

b' Dissected embryo showing two cotyledons (Cot) and suspensor haustorium (Sh). Note the embryo is greenish.

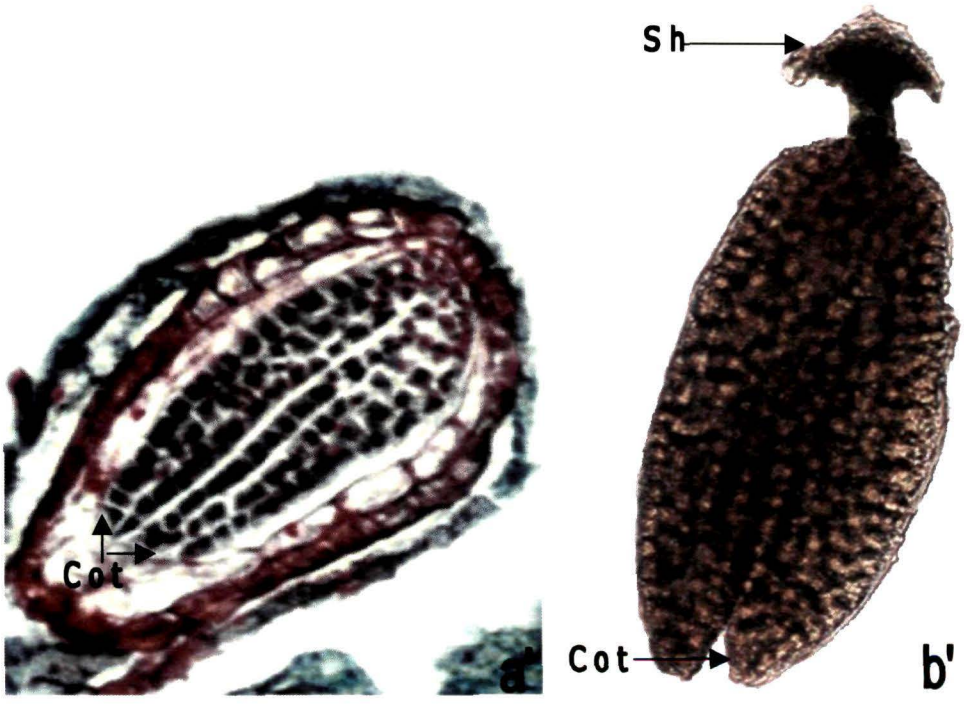
(x= x 1000; y& z= x 1500; a'= x 450; b'= x 400)

Plate- 6.4

***Podostemum subulatus* Gard.**



Elongated torpedo stage Section of mature embryo



L.S. of mature embryo Chloroembryo

CHAPTER - 7

ECOLOGY OF PODOSTEMACEAE

7.1 *Introduction*

Podostemaceae prefers such a habitat, unusual and remarkable for any family of angiosperms: tenaciously attached to substrata in torrential currents of waterfalls and river-rapids. They are reported to occur only in the areas of rapids, where they tolerate current speeds of 2-3 $m.s^{-1}$ (Neiff, 1986). Various authors have suggested that water turbulence provides an aeration needed for uptake of gas by these plants (e.g. Gessner & Hammer 1962; Grubert 1976; Sculthorpe 1967). The nature of habitat is associated with unusual morphology and phenotypic plasticity (Rutishauser 1997).

The plants are haptophytes (Luther 1949; Cook, 1996), i.e., plants that attach to, but do not penetrate, a solid substratum. The substratum is typically a rock. Specific rock type varies depending on the geographical region, e.g., granite, basalt, manganese or various sedimentary rocks. Podostemaceae are rare in limestone regions. It is unclear whether the nature of substrate or water chemistry (or combination of both) limits the occurrence of Podostemaceae in these regions. The plants typically occur in rivers of low nutrients

contents, although little is known about their specific nutrient requirements. Podostemaceae predominate in areas of high light intensity, i.e., unshaded areas. The largest population of most species occur in areas of full sunlight.

Podostemad life cycles are linked to the hydrological cycle of their habitat, i.e., the high and low water periods. The vegetative plant body grows submerged during the rainy season, flowering is initiated when the level of water drops and plants are exposed. Flowers develop inside a sac-like covering (spathella) and opens as the spathella ruptures. Philbrick (1984), however, reported an atypical instance the spathella of *Podostemum ceratophyllum* could rupture on the thallus that are up to 26 cms below the water surface.

Populations demonstrate an annual cycle of colonization, canopy establishment and die back. For instance in *Podostemum* and some other perennial species, the prostrate roots that remain wet (e.g., on lower areas of rocks) during the low water season, initiate growth when water level rises.

All these unusual and typical characteristics necessitate the examination of habitats, physico-chemical properties of water which restrict the plants to only very few sites in North Eastern India. Therefore, this chapter describes the habitat of this enigmatic family

and elucidates physico-chemical properties of Janiaw river (*Hydrobryum griffithii* and *Polypleurum wallichii*) and Umtienger river (*Podostemum subulatus*).

7.2 *Observation*

The chemical analysis of water collected from the stream near Umtienger (91° 70'N – 25° 40'E), about 22 kms away from Shillong, harboring *Podostemum* is given below.

Table-7.2 (a) Site-I (Umtienger).

PROPERTIES					
Sl.	**Parameters**	**Result**	**Sl**	**Parameter**	**Result**
1	Temperature	9°C	6	Chloride (mg/l)	10.65
2	pH	6.84	7	B O D (mg/l)	4.0
3	Alkalinity (mg/ l)	26.0	8	NO_2 (mg/l)	0.009
4	Total hardness (mg/l)	2.0	9	PO_4 (mg/l)	0.004
5	Dissolved O_2 (mg/l)	11.60	10	SO_4 (mg/l)	1.088

(Results are average values of 3 months data from October to December)

Chemical analysis of water, conducted using the same parameters in a stream at fossil park, Janiaw, Lawbah region (91° 50'N – 25° 20'E); about 6 km away from Mawsynram, inhabited by *Polypleurum* and *Hydrobryum.*

Table-7.2 (b) Site-II (Janiaw, Lawbah).

PROPERTIES					
Sl.	**Parameters**	**Result**	**Sl**	**Parameter**	**Result**
1	Temperature	10°C	6	Chloride (mg/l)	10.80
2	pH	8.68	7	B O D (mg/l)	2.0
3	Alkalinity (mg/ l)	88.0	8	NO_2 (mg/l)	0.007
4	Total hardness (mg/l)	0	9	PO_4 (mg/l)	0.156
5	Dissolved O_2 (mg/l)	6.5	10	SO_4 (mg/l)	2.902

(Results are an average values of 3 months data from October to December)

7.2.1 *Temperature and pH*

The temperature of water in the sites of samples collected during the period of investigation is observed which ranges from 9° C - 10° C respectively. The samples from site-I have pH of 6.84

containing no carbonates but bicarbonates and carbonic acids. Therefore, *Podostemum* is growing in slightly acidic habitat; in other words, it is more or less free from the acidic condition of water.

On the other hand, site-II have a pH of 8.68 containing carbonates with or without bicarbonates; they don't have free carbonic acids. It is observed that *Hydrobryum* and *Polypleurum* prefer slightly alkaline waters. The pH data show that the waters are slightly basic and are attributed to less human activities such as biological and chemical waste disposal.

7.2.2 *Total alkalinity*

The determination of total alkalinity (TA) gives an idea of salt present in water samples. In general, if alkalinity is greater than hardness, in addition to calcium and magnesium salts, basic sodium and potassium salts are also present.

From the tables 7.2 (a) and 7.2 (b), total alkalinity far exceeds the total hardness in both the sites, viz. 26:2 in site-I and 88:0 that indicates that the water is very rich in salt contents.

7.2.3 *Cl^-, NO_2, , PO_4 and SO_4 contents*

The data in table 7.2 (a) and 7.2 (b) show the NO_2, PO_4, SO_4 and Cl^- concentrations. Cl^- content is considerably high in both sites; whereas the amount of NO_2 and PO_4 present are negligible when

compared to SO_4 content. NPK is an important constituent for the growth of plants. Though K can be derived from water in the form of carbonates; the dissolved PO_4 and NO_2 are not significant. As a result, the plants depend upon other sources for their growth.

7.2.4 *Total hardness*

Total hardness (TH) is defined as the reluctance of water to give lather with soap. According to the classification of water based on total hardness, waters with hardness below 50 are soft water; the two study sites have very low total hardness i.e., 2.0 and 0.0 respectively. Since calcium and magnesium are the principal cations causing hardness, the two study sites are very poor in calcium and magnesium contents. These plants grow where the total hardness of water do not exceed 5.0.

7.2.5 *Dissolved Oxygen*

Dissolved oxygen is a measure of the corrosiveness of water, photosynthetic activity and septicity of water. Respiration by plants and animals reduces oxygen concentration, while photosynthetic activity of plant increases it. The dissolved oxygen is considerably high in the two sites, 11.6 and 5.5 respectively. Since, these plants lack intercellular spaces to maintain gaseous oxygen, well-oxygenated water is essential for their growth.

7.2.6 *Biological Oxygen Demand (BOD)*

Biological oxygen demand is a test of great value in the analysis of sewage and grossly the extent of pollution. The two sites have BOD of 4.0 and 2.0 mg/l respectively. As per the Royal Commission classification, Site-I falls under "Fairly Clean" whereas Site-II with a BOD of 2.0 is regarded as "Clean" with lesser influence of pollution. The investigation reveals that some members viz., *Hydrobryum, Polypleurum* and *Podostemum* inhabit clean and unpolluted water.

CHAPTER – 8

GENERAL DISCUSSION

8.1 Morphology

Podostemaceae is the largest family of strictly aquatic angiosperm with 49 genera and 270 species worldwide (Philbrick & Novello, 1998); about 11 genera and 42 species are reported in India, (Hooker, 1885; Cook, 1996; Mohan Ram & Anita Seghal, 2001; Mathew, 2003).

The members of the family are commonly called river-weeds with very peculiar vegetative form; revealing many unique morphological, anatomical and ecological features and stands clearly apart from all other angiospermous family (Willis 1902, Schnell 1967, Nagendran et al., 1977, Rutishauser, 1995). The architecture of the plant body deviates remarkably from the root-shoot system common in angiosperms (Rutishauser, 1997, 1999).

In this thesis, the morphological characters are rather employed to delimit species than reproductive characters; because the reproductive parts show much similarity except for a few characters like sizes and no. of ridges on the capsule, locules of capsules (isolobous in *Polypleurum* and *Hydrobryum,* whereas it is anisolobous in *Podostemum*). The number of stamens and

staminodes; number of stigmatic lobes are almost same in all the three species studied. The three shows much vegetative variation than reproductive characters. The shape of the thalloid plant body has been found to be constant in each species. The thalloid plant body is believed to be of more taxonomic value than the floral characters. In H.*griffithii*, the thallus is small, flat; more or less circular in outline, coriaceous green with filiform leaves, closely attached to substrate. Whereas in P. *wallichii*, thallus is lobulate with undulate margin, which grow erect; the venation is pinnate; the flower arise on the margins of frond. In P.*subulatus*, the growth form of the thalloid plant body is thread like, with alternate leaves.

There is evidence that the plumule aborts rapidly in seedlings, in which case the thalloid growth form could represent a highly specialized photosynthetic root system (Domer, 1972; Fahn, 1972). Some features of riverweed are so extensively modified that they superficially resemble monocotyledon characteristics. The presence of silica bodies, trimerous gynoecium, and fused stamen filaments are reminiscent of conditions found in the highly specialized monocotyledon family Orchidaceae (Dahlgren, 1980). The androecium is made up of a pair of laterally placed staminodes and generally two fertile stamens fused at the base.

The zygomorphic flower possesses a bi-carpellate ovary with two stigmatic lobes; two stamens at the top of an androphore and two filiform tepals, basally at both sides of the androphore. Philbrick (2004) used the terminiology tepals for staminodes. The length of androphore and tepals relative to the ovary varies within many species (Nileena, 2001). In most species floral features are therefore of little taxonomic value. The variation in the floral structure has led to the conclusion that many of the hitherto described species may be phenotypic variations of a single species (Rutishauser, 1997,1999). As is typical for Podostemoideae each flower is covered by a thin clavate spathella. When each pedicel grows pushing up the flower, it splits the margins of spathella and emerges.

In the present study the plumule and radicle are aborted during early embryogenesis. The thallus is an outgrowth from cotyledon as well as hypocotyledonary region. Rhizoids develop from the base of hypocotyls and at the same time the thallus also regenerate from the cotyledons. Because the cotyledons at the time of differentiation undergo dorsiventral symmetry, the thallus cannot be regarded as root as reported by Cook (1996)

8.2 Anther development and microsporogenesis

Tetrasporangiate anthers have been reported in majority of angiosperm families (Bhandari, 1984). In *Hydrobryum griffithii*,

Polypleurum wallichii and *Podostemum subulatus* (Podostemaceae), the two staminal primordial arise independently; because of the intercalary growth of andropodium, it becomes bifid or forked. The two forked stamen exceeding the ovary and lie close to the stigma. Intercalary growth of andropodium has also been reported in N. *khasiana* and N. *lowii* (Venugopal & Rashi devi, 2003). In all the plants studied, the four microsporangia are protuberant on the adaxial surface; a similar situation is reported in Anonaceae, Degeneriaceae and Hemantandraceae except that the microsporangia are embedded and not protuberant (Bhandari, 1984).

A plate of 3-4 cells in *Hydrobryum* and *Polypleurum*, but 4-6 cells in *Podostemum*, of hypodermal origin function as archesporial initial. A similar type of hypodermal cell has also been reported in many plants (Maheshwari, 1950; Perisamy & Swamy, 1959; Perisamy & Kandasamy, 1981). In Indotristicha ramosissima (Wight) Van Royen and Terniola zeylanica (Gardn) Tulsane, both belonging to the family Tristichaceae, showed a group of archesporial initial of hypodermal origin (Mukkada, 1969; Chopra & Mukkada, 1966) during anther wall development. Kapil (1970) reported the differentiation of middle layer(s) from the secondary parietal layers.

However, the present study reveals that the anther wall consist of only endothecium and tapetum. Middle layer is absent in all the plants studied (viz. *Hydrobryum, Polypleurum* and *Podostemum).* The pro-orbicules or Ubisch bodies appear in the tapetum by the time when the microspore mother cells completed meiosis-I and enter into the meiosis-II in all the three plants. As soon as cell division is completed, these granules are released in the anther locules; engulfed and deposited on the tetrads.

In all the three plants, the entire tapetum arises from the parietal tissue as a concentric layer around the sporogenous cells. A similar type of origin of tapetum exclusively from the parietal layer has been reported in *Triticale* (Bhandari & Khosla, 1982). The tapetum is of secretory type, unilayered structure; cells with distinct uni and bi- nucleate condition and dense cytoplasm that persists until the formation of dyad microspores. The anther wall development belongs to reduced type (Davis, 1966; Poddubnaya-Arnoli, 1976).

The sporopollenin deposited on the pollen grain as tiny granulation is mainly secreted by tapetum in all the three plant studied. The secretory type of tapetum has been observed in *Indotristica, Terniola, Dicraea stylosa* (Mukkada, 1969), *Griffithella* (Razi, 1949); *Podostemum*, *Hydrobryum* (Magnus, 1913) and

Angolea (Went 1910, 1912). Echlin (1971) and Shaw (1971) also reported the tapetal origin of sporopollenin in *Lilium henryi*.

In *Terniola, Indotristicha (*Mukkada, 1966, 1969; Chopra& Mukkada 1966; Kapil, 1970) reported that the pollen mother cell undergoes simultaneous type of cytokinesis. In the present study, the pollen mother cell division is of successive type. The starch grains in the endothecial layer as well as adjacent connective tissues have been utilized for the development of pollen grains. Similar observation was observed in *Lilium* (Reznickova & Williemse, 1982).

Meiosis is not uniform in all the four thecae of an anther lobe. This feature has not been recorded so far in any member of Podostemaceae. The cell division in the three plants follows successive type in which the meiosis-I is followed by centrifugal cell plate formation, after which the two daughter cells are enclosed by distinct callose wall that the resultant cells lie side by side in the common callose wall. The callose wall acts as a barrier or semi-molecular filter to allow only some macromolecules as well as providing genetic autonomy to each developing sporocytes (Heslop-Harrison & Mackenzie, 1967). Meiosis-II takes place in the resultant daughter cells so that two dyads are formed. After which, callose wall starts to disappear. Meiosis-I and II are two different

phenomena, not interconnected one another, so that two genetically different dyads are formed.

Perisamy and Swamy (1959) emphasized that the terminology successive and simultaneous types should not only represent temporal relationship, but two different cytological processes; the former is affected by cell plate while the latter by furrows. In successive type, the dissolution of callose wall after the formation of four microspores has been reported in *Pooea purpuria* and *Laventera trimestris* (Longly & Waterkeyn, 1979). The parenchymatous cells adjacent to the anther locule to acquire endothecial thickening is also seen in *Chelone globra* (Arekal, 1963). Subramanyam and Shreemadhavan (1969) reported that anthers dithecous and anther wall four layered in both the Podostemacaee and Trictichacaeae. The present investigation did not confirm this view.

Pollens *Hydrobryum*, *Polypleurum* and *Podostemum* are relatively small, spherical and microechinate. In term of structure, grains of all three taxa have a tectate-granular sexine and a thick nexine in non-apertural region. Pollen grains of some members like *Marathrum rubrum*, *Oserya coulteriana*, *Tristicha trifaria* and *Vanroyenella plumose* are shed as monads whereas grains of *Hydrobryum griffithii, Polypleurum wallichii* and *podostemum subulatus* occur in well defined dyads. Dyad pollen grains are the

characteristic feature of the sub family podostemoideae (Rutihauser, 1997). Granules in pollen wall of *Marathrum*, *Podostemum*, *Tristicha* and *Vanroyenella* are relatively uniform in size and minute, whereas those making up of the infratectum in *Oserya* pollen are considerably larger and irregularly shaped. Pollen grains must be among the shortest-lived independent bodies in nature, for there are few which can remain alive for more than a few days after they have been shed, while some can live for only a few hours (Echlin, 1968; Sporne, 1974). At the time of anther dehiscence, the pollen grains of most flowering plants are bicellular; they contain vegetative cell and generative cell. Nearly 75% of flowering plants studied shed pollen in bicellular stage, and 25% released pollen as tricellular grains following sperm production (Brewbaker, 1967; Raghavan, 2000).

Understanding of various factors influencing pollen germination and tube growth is prerequisite for the success of hybridization program (Vasil, 1964; Mercy et al., 1978). Viable pollen will help in giving successful result for plant breeders in breeding programme, which requires huge expenditure of time as well as money. Pollen viability should be considered different from germination, for instance, self or cross incompatible pollen, though viable may fail to germinate due to lack of certain essential factors (Goswami, 2002). It is however necessary that viable pollen

should give high percentage of germination for guessing effective fertilization (Semalty & Sharma, 1996).

In the present study, *Hydrobryum* and *Polypleurum* show high pollen viability (73.68% and 82.60% respectively) whereas *Podostemum* shows considerably low viability (36.17%). In all the angiosperm species the pollen viability decreases with time (Pacini et al., 1997). Binucleate pollen generally survives longer than trinucleate and germinates readily in vitro (Stanley and Linskens, 1974). Entomophilous species also generally have longer pollen viability (Pacini et al., 1997). But in Podostemaceae the pollens seem remain viable for only a short period. This suggests that the pollen grains are in a highly active metabolic state and, as a result, the biochemical changes takes place over time and one could expect more viable seeds with high pollen viability.

8.3 Megasporogenesis and Embryo sac development

Since the beginning of 20th century it has been known that species of Podostemaceae possesses a four-celled embryo sac, rather than 8-celled as is more common in angiosperms. In this study, I have found three embryological characteristics that consistently distinguish Podostemaceae from other angiosperms: 1) a tetra cellular embryo sac reduced to a micropylar quartet; 2) The occurrence of a nucellar plasmodium enclosed within a cuticle

and endothelium; 3) Absence of endosperm formation, an indicative for the lack of triple fusion.

Embryology of several Indian species of Podostemaceae has been investigated (Razi, 1949, 1955; Maheswari, 1955; Mukkada, 1962, 1966; Kapil, 1970; Battaglia, 1971; Mukkada & Chopra, 1973; Arekal & Nagendran, 1974, 1975a,b, 1976; Nagendran, 1975; Nagendran & Arekal, 1976; Nagendran et al., 1981; Johri et al., 1992; Subramanyam & Sreemadhavan, 1969). Because the female gametophyte is highly reduced in structure (See Battaglia, 1971; Arekal & Nagendran, 1975b, 1976). Mukkada (1962, 1969) traced the embryogeny in *Terniola zeylanica* (Gardner) Tulsane, *Indotristicha ramosissimma* (Wight) Van Royen and *Dicraea stylosa* Wight. Nagendran et al., (1981) studied the development of female gametophytes in *Hydrobryum griffithii* (Wallich ex Griffith) Tulsane.

Two modes of four-celled embryo sac formation are encountered in the three plants studied; bisporic type in *Polypleurum* and monosporic development in *Hydrobryum* and *Podostemum*. The most common type of embryo sac in the family seems to be the *Apinagia* type, although the details of embryo sac development in some species are unclear and / or controversial (Nagendran, 1974). An inverted organization of embryo sac is reported by Arekal and Nagendran (1976) in *Willisia selaginoides*.

Jager zurn (1967) discussed many of the issues that remain unresolved regarding embryo sac development in Podostemaceae.

In the present study, the embryo sac organization of *Hydrobryum griffithii* and *Podostemum subulatus* corresponds to Apinagia B (Battaglia 1971) and *Polypleurum wallichii* conforms to both *Dicraea* type and *Podostemum* type. The *Podostemum* pattern of development has largely been controversial because, unlike other types, it does not exist independently in any of the species studied. In these species, the micropylar cell of dyad degenerates and persist only as a crescent-shaped (pycnotic) cell while the chalazal cell of the dyad completes meiosis-II; But as the chalazal nucleus degenerates in the dyad, only the micropylar nucleus is functional in the formation of final embryo sac structure; according to Maheshwari (1937) and Nagendran (1974), the type of embryo sac in *Podostemum* and *Hydrobryum* are monosporic in origin since only the micropylar nucleus of the chalazal dyad cell is functional in embryo sac formation. This micropylar nucleus in the chalazal dyad cell undergoes two cycles of mitoses and produces four uninucleate cells. From biological point if view, the chalazal nucleus does not contribute to the subsequent formation of female gametophyte (Maheshwari, 1955; Arekal & Nagendran 1975a) and henceforth does not contribute to the genetic variability of the embryo sac.

However, the micropylar dyad and chalazal dyad are active in embryo sac formation conforming to bisporic type in *Polypleurum wallichii*, which divides only once to form four-nucleate embryo sac. The egg cell is in the median position, located below the synergids and a chalazal polar nucleus. Interestingly, occasional organizations, wherein the synergids occupy the chalazal pole with the polar nucleus at the micropylar end have been observed (Arekal & Nagendran, 1976).

In some species, two synergids in the egg apparatus degenerate before the arrival 0f pollen tube or right after pollen tube enter into the embryo sac or before fertilizationl (Battaglia, 1971; Nagendran *et al.*, 1976, 1980); in others in which the egg is flanked by only one synergid, the remaining two nuclei are designated as antipodal (Mukkada, 1963, 1964; Arekal & Nagendran, 1975). This implies that the absence of a true polar nucleus in the embryo sac precludes fusion of the second male gamete initiating another fertilization event and formation of the endosperm. Understanding the reasons for the absence of double fertilization in this family is a real challenge because mechanical factors such as the failure of the pollen tube to discharge the second sperm are also probably involved (Chopra & Mukkada, 1966; Mukkada, 1969). The limited contributions on the reproductive biology of basal angiosperms that are currently available pertain mostly to descriptive accounts of their floral

morphology and comparative embryology (Friedman, 2001a; Friedman & Floyd, 2001).

The development and organization of embryo sac in *Hydrobryum griffithii* conforms to the observation of Nagendran et al., (1976). In angiosperms, only three families viz. Podostemaceae, Orchidaceae and Trapaceae do not have endosperm (Vijayaraghavan & Prabhakar, 1984). In Orchidaceae and Trapaceae the triple fusion occurs in general but primary endosperm nucleus undergoes only few divisions and subsequently degenerated (Cocucci, 1964; Tohda, 1967; Ekanthappa & Arekal, 1977a; Johri et al., 1992). However, in Podostemaceae, triple fusion does not takes place at all, therefore the formation of endosperm is absent, which is an exclusive feature of the family Podostemaceae (Kapil, 1970b; Mukkada, 1962b; Chopra & Mukkada, 1966; Mohan Ram & Anita Sehgal, 2001).

8.4 *Embryogeny*

As soon as the syngamy is over, the polar nucleus begins to degenerate; the zygote increases in its volume before embarking on division. Similar feature has been observed in orchid *Cypripedium* (Poddubnaya-Arnoldi, 1967) and in the members of Lentibulariaceae and Orobanchaceae (Natesh & Rau, 1984). This phenomenon can be attributed as to push the future embryonal

pole into nutritionally more favorable medium i.e., nucellar plasmodium as suggested by Natesh and Rau, (1984). The zygote of three specimens shows distinct polarity of its distribution of organelles such as nucleus and starch grains. This feature is a universal phenomenon among angiosperms that the zygotes exhibit apicobasasl polarity (Jensen, 1968; Raghavan, 2000; Mansfield & Briarty, 1991).

On the embarkment of first division of zygote, the two cells 'ca' and 'cb' are formed. The cell 'ca' becomes richly cytoplasmic, with distinct nucleus than the 'cb' (Swamy & Krishnamurthy, 1980; Schultz & Jensen, 1968; Raghavan, 2000). Alvarez and Sagawa (1965) distinguished the differences between 'ca' and 'cb' by histochemical methods. They reported more RNA and Protein in 'ca' than in 'cb'. Therefore, these two daughter cells inherit differentially distributed cytoplasmic elements. It shows that the major portion of the future embryo is formed mainly from 'ca' (Natesh & Rau, 1984).

The polarity and differential distribution of cellular organelles and starch grains in the derivatives of zygote in shows that the potential embryonic axis pushes towards the nutritionally favorable pole i.e., nucellar plasmodium. The octant stage gives important clues for functional differences between the superposed tiers of cells because the upper tier contributes to the cotyledons while the lower one is the source of hypophyseal region. Similar

feature has been reported in Cotton and many other plants (Jensen, 1968, Raghavan 2000). Previous workers (Maheswari, 1950; Subramanyam & Sreemadhavan, 1969; Kapil, 1970; Mukkada, 1969; Mukkada & Chopra, 1973; Johri et al., 1992; Jager- Zurn, 1997; Mohan Ram & Anita Sehgal, 2001) reported only the solanad type of embryogeny in Podostemaceae, but the present investigation revealed the Onagrad type of embryo development in *H. griffithii* and *Podostemum subulatus* which has not been recorded so far in any members of Podostemaceae, whereas *Polypleurum wallichii* follows solanad type of embryogeny. The onagrad type of embryogeny and the presence of endothelium in H. *griffithi* and *Podostemum subulatus* show close relationship with Hydrostachyaceae than the Crassulaceae. (Jager-Zurn, 1965; Maheswari, 1948).

8.5 Nutrition of the embryo

The Podostemaceae uniquely possesses a 'pseudo embryo sac' also called nucellar plasmodium, which results from disintegration of nucellar cells to form multinucleate plasmodial structure just below the embryo sac. Because there is neither double fertilization, nor endosperm development in Podostemaceae (Battaglia, 1980), it is assumed that the nucellar plasmodium nourishes the developing embryo (Davis 1966; Arekal & Nagendran 1975a) and protects it from desiccation (Magnus

1913). Clearly there is a wide range of variation in the initiation stage of nucellar plasmodium development inside podostemaceaen sub families. In sub-family Podostemoideae a nucellar plasmodium develops prior to fertilization (Nagendran et. al., 1977) whereas in sub-family Tristichoideae it occurs only after fertilization (Arekal & Nagendran 1977b).

Although in sub-family Podostemoideae, nucellar plasmodium formation starts before fertilization, the precise stage varies among species. The more common report is for the nucellar plasmodium to begin development at the two nucleate embryo sac stage, as occurs in *Hydrobryum griffithii* (Nagendran et al 1976), *Polypleurum filifolium* P. *dichotomum* (Nagendran et al (1977) and *Zeylandium olivaceum*, Z. *johnsonii* (Arekal & Nagendran 1977a). In contrast, it begins to develop at the time the megaspore mother cell undergoes meiosis-I in *Podostemum subulatus* (Nagendran et al, 1981); at early meiosis in *Dicraea stylosa* (Mukkada 1962) and after meiosis-I in *Vanroyenella plumose* (Sanchez et al., 2002) and the stage with the embryo sac is mature in the Asian *Lawia zeylanica* (=*Dalzellia*) (Razi 1949). In sub-family Tristichoideae, nucellar plasmodium formation also occurs at varying times, although in all cases, it is after fertilization. However in both the patterns, the disintegration of the cell walls between the nucellar cells lead to the fusion of uninucleate protoplast that has been

referred to as pseudoembryosac by Went (1910), Magnus (1913) and others.

Another remarkable feature of the family is possession of suspensor haustoria. The main function is to anchor the pro embryo to the embryo sac wall and facilitate the translocation of nutrition to the embryo from the surrounding tissues. The structure of suspensor haustorium in *Hydrobryum griffithii* looks like *Halogris micrantha*, but in latter haustorial branches are absent. In *Dicraea stylosa*, the two nuclei are superposed in a single cell, on the other hand in *Hydrobryum griffithii*, the two nuclei are hypertrophied and located in juxtaposed position.

Endothelium (or integumentary tapetum) is invariably associated with unitegmic, tenuinucellate ovules. The presence of endothelium is recorded in 65 families of dicotyledons (Kapil and Tiwari, 1978). In Podostemaceae, the inner integument is initiated after the complete development of the outer integument (Bouman, 1984; Mukkada, 1962a, 1964; Arekal and Nagendran, 1976). In H. *griffithii*, the inner integument consists of two layers of cells, out of which the inner layer adjoining with nucellar plasmodium is transformed into integumentary tapetum. The cells are elongated and perpendicular to the embryo sac. The cell walls of endothelium bear wall projections, these wall ingrowths similar to that of transfer cells (Gunning and Pate, 1974). In *Hydrobryum*

griffithii the endothelium is restricted around the middle portion of the nucellar plasmodium. Similar feature has been recorded in *Linaria ramosissima* (Arekal and Raju, 1964) and *Scoparia dulcis* (Arekal et al., 1971).

Kapil (1970), Mohan Ram and Sehgal (2001), Kapil and Tiwari (1978), Mukkada (1962b, 1964); Arekal and Nagendran (1975a), Nagendran et al. (1981), Battaglia, (1971) have studied the extensively the reproductive biology of Podostemaceae, but they have not mentioned anything about the presence of endothelium in Podostemaceae. This is the first record of the presence of endothelium in *Hydrobryum*, *Polypleurum* and *Podostemum* and its nutritional role for the development of embryo.

8.6 *Biology of the plant*

Though general account of the biology of Podostemaceae have been published since the early studies of Willis (1902), Went (1929) and many others, the detail of virtually every phase of life-cycle remain poorly unknown, in large part because of the extreme habitat in which they occur.

Podostemaceae are the only macrophytes that dominate river rapids and waterfall habitats. Plant succession (i.e., directional change in species composition over time) does not occur among macrophytes in these chronically disturbed habitats.

In the three plants studied during the investigation, the population of the same species recolonize the same habitats each successive years. The population undergoes an annual cycle of colonization, establishment of a canopy of mature plant, and die back when the water level drops. The annual pattern is dictated by high and low water periods. Species life cycles are closely tied to coping with these extremes and the intermediate conditions that connect them temporally.

The members of the family superficially resemble an algae or bryophyte; distinguished by the presence of flowers or fruits in the leaf axils (See illustration in Godfrey & Wooten, 1981). The conventional demarcation into stem, leaf and root is often lacking or at least not obvious (Nagendran 1983; Cusset &Cusset, 1998; Mohan ram & Sehgal, 1992; Rutishauser, 1997).

The vegetative plant body reflects apparent adaptation to the severe mechanical stress imposed by swift current i.e., leaves are often highly dissected and caduceus. Many authors have discussed the extreme structural modifications that have accompanied the invasion of aquatic environment by terrestrial plant group (Arber, 1920; Sculpthore, 1967). Such modifications are particularly striking in Podostemaceae. Riverweed adaptation has resulted in an unorthodox morphological organization, therefore, the authors have described as 'alga-like', 'fucoid',

lichen-like, moss-like or webbed (Graham & Wood, 1975; Cronquist, 1981; Van steenis, 1981)

Podostemaceae attach to the substratum by rhizoidal hairs (adhesive hairs) and holdfast-like structures called haptera. Rhizoids occur on the undersides of roots, both at the growing apices and along older sections and stems (Philbrick 1984; Rutihauser 1997; Jager Zurn & Grubert 2000). Various authors have reported that glue like substance secreted from rhizoidal hairs is a primary means of attachment (Engler 1930; Vidyashankari 1988; Rutihauser 1997).

It has long been recognized that concomitant with clonal growth in aquatics is a reduction (or loss) of sexual reproduction (Barrett et al., 1993; Grace, 1993; Les, 1988; Sculpthore, 1967). The Podostemaceae often contradict these general patterns in aquatics. They are strongly attached to the substratum; lacks the various types of modified vegetative structures (e.g., winter buds, stem fragments) that promote asexual reproduction and dispersal in other aquatics. Indeed when we consider many traits associated with aquatics overall, Podostemaceae seem more terrestrial than aquatics.

In Podostemaceae, anthesis is generally believed to take place only after the flower buds are exposed by the dropping water level (Accorsi, 1953; Royen, 1951; Went, 1929; Willis, 1902a,b). Flower develops inside a sac-like covering (spathella) and open as

a spathella ruptures. In the present investigation, Hydrobryum, Polypleurum and Podostemum also confirm that the sexual reproductive structures expanded when the water level recedes. Philbrick (1984) however reported that the spathella of P.*ceratophyllum* could rupture on stems that are up to 26 cms below the water surface. The regularity of annual flowering combined with copious seed production contrasts markedly with the apparent reduced role of sexual reproduction in aquatic plants in general (e.g., Sculpthore, 1967). Indeed sexual reproduction may have played a central role in the evolution of Podostemaceae. Reproduction via seed is of primary importance in the biology of Podostemaceae.

There is no evidence to suggest water pollination (hydrophily) in any one of the three specimens studied. The flower lacks essentially all the features attributed to hydrophily and there is no close association between flowers and the water surface. It is skeptical to see the report of Hall (1971) that an African species, *Polypleurum submersum* is pollinated by pollen transported across the water surface. The overall floral structure (i.e., lack of showy petals, nectars or other possible rewards) supports my supposition that biotic pollination does not occur. Yet the large number of ovules per ovary and lack of copious pollen production (i.e., relatively low pollen-ovule ratios) is not typical for wind pollination (Faegri & van der Pijl, 1979; proctor et al., 1996;

Philbrick & Novello, 1998). On the contrary, Entomophilly, anemophily, and hydrophily have all been reported in the family (Hall, 1972; Sculthorpe, 1967; Went, 1929; Willis, 1902b). However, based on floral morphology and field observation, anemophily appears to be the primary pollination system.

Seeds of the three specimens typically germinate on solid substrates, often granite. Seedling with two cotyledons and adhesive hairs as hypocotyls outgrowths, as is typical for Podostemaceae was also documented during the exploration. Germination occurs only when seeds are submerged (i.e., when the water level has risen).

Unlike many other aquatic flowering plants, I do not encounter any asexual propagules of dispersal. Colonization by clonal growth only occurs from plants already present on the rock. In contrast, colonization of new rock, other cataracts, or other river is possible via seeds. Perennial Podostemaceae seem similar to other aquatic angiosperms that rely on clonal growth for maintenance of local populations, but depend on seeds for dispersal to new locations (Philbrick & Les 1996).

Seed dispersal vectors are not known for any Podostemaceae, but biotic (e.g. Birds) and abiotic (wind, water) agents are possible (Engler, 1930; Grubert, 1975, 1976). Birds are seen on rocks around river-rapids where Podostemaceae occurs. Seeds are small; numerous seeds produced per capsule. It is

conceivable that birds walking on rocks where seeds have been shed could pick them up on their feet. Seed transport on the water surface is also likely.

Because the seed lacks morphological and anatomical structures that enable them to stay afloat, they do not seem superficially adapted for water transport (Willis 1902a). Willis considered these seeds to be terrestrially adapted although he said little about their dispersal capabilities

The outer integument of seed plays a central role in the attachment of seed on the solid substrate (e.g. rocks) (Accorsi, 1953; Gessner & Hammer, 1962; Grubert 1970, 1975, 1976; Philbrick 1984). Upon wetting, however, these cells take up water rapidly, expand and become mucilaginous (sticky). When a wetted sticky seed comes in contact with the rock, the outer integument adheres the seed. Grubert (1970, 1980) in his studies of *Mourera fluviatilis* Aublet, reported that the adhesive strength of the mucilage far exceeds the shearing forces of the water current. He also illustrated that the development of mucilaginous layer is sensitive to pH of water.

8.7 *Ecology of the family*

Plants in the wide spectrum of distribution in the old and new world family Podostemaceae, inhabit rocks in turbulent waterfalls and cascades. All genera of Podostemaceae appear to

require the same five general ecological conditions: Solid substrata, seasonal water level periodicity, swift clean water, high light intensity, a biofilm of cyanobacteria.

Rock surface to which plants attach vary in texture from smooth to rough and in orientation from vertical faces of waterfalls to horizontal or irregularly oriented outcrops. Philbrick and Novello (2004) reported some members growing attached to non-rock substrata (e.g., tree trunk and branches), including man made objects (e.g. concrete pilings, automobile hoods, steel shopping baskets). Thus, a primary factor for attachment seems to be substrate stability (lack of movement), rather than the nature of substratum itself. Various authors have reported that a glue-like substance secreted from rhizoidal hair is primary means of attachment (Engler, 1930; Vidyashankari, 1988; Rutihauser 1997).

An important discovery concerning the attachment of plants and overall ecology of Podostemaceae was reported by Jager-Zurn (1999) and Jager- Zurn and Grubert (2000). These authors documented the presence of bacterial biofilm layer between the rhizoidal hairs of plant and the substratum. They presented compelling evidence to support their argument that cyanobacteria played an important role in the attachment of Podostemaceae. The bacterial biofilm may represent an important source of nitrogen for these plants, many of which are restricted to low nutrients.

In the present study, different physico-chemical parameters were analyzed and correlated with the ecology of three specimens studied. Total hardness is very low (2.0 and 0 respectively in site-I and site-II) in contrast to the rivers of Kerela, where it ranges from 10-20 (Mathew et al., 2003) and black water rivers of Venezuela examined by Gessner and Hammer (1962), as well as some rivers of Mexico (Quiroz et al., 1997), indicating low content of calcium and magnesium. Interestingly also, pH of the study sites vary from those of New world, 6.84 and 8.68 respectively; but ranging from 7.1 to 9.1 in Mexican rivers and 4.7- 4.8 in the black water river Caroni. The two study sites having high pH indicates less human activities of different kind. Philbrick & Novello (2004) reported that the members are more common in rivers with clean water than those that are polluted. Podostemaceae predominate in areas of high light intensity i.e. unshaded areas. The largest population of the species is observed to occupy an area of full sunlight.

With the assays of BOD, it is well established that *Hydrobryum griffithii, Podostemon subulatus* and *Polypleurum wallichii* inhabit clean rivers with less pollution and human influences. Meijer (1976) suggests that the occurrence of riverweed is an indicator of good water quality. The family therefore serves as an indicator of clean water.

However, the content of dissolved oxygen in the water of two streams examined are quite high and falls in the same range with Kerela rivers and of Rio caroni, Venezuela (6.5 mgl^{-1}- 11 mgl^{-1}). The rivers in the study site I & II are fast flowing and well oxygenated; the young plants are subjected to high water flow pressure; dim light, low CO_2 and high dissolved O_2. The result shows that the dissolved oxygen (and carbon dioxide dissolved in water) is apparently the most essential factor for the successful establishment of these plants, rather than it is the content of other nutrients and the pH. At the same time, it is also established that Podostemaceae involve much in photosynthetic activity and biochemical processes. A lack of intercellular spaces in the plant body of Podostemaceae (Arber 1920, Metcalf & Chalk 1950; Sculthorpe 1967) likely is associated with the highly aerated water in which they occur.

Pannier (1960) reported that *Tristicha trifaria* (Bory ex willd.) Spreng. is unable to use bicarbonate. Thus the principal carbon source available for photosynthesis may be free dissolved CO_2, which is more available at lower pH (Wetzel 1983). Limited free CO_2 availability may relate to the paucity of Podostemaceae in limestone region. During periods of low water in some instances, plants can persist in areas of little current, e.g., quiet pools along the margins of river-rapids. Willis (1914) reported that some Ceylon (Sri Lanka) species occur in relatively placid water current.

Irang et al. (2003) reported the presence of *Podostemum rutifolium* (as P. *fruticulosum*) on rock outcrops partially buried in sand along the wave-swept beach of Brazilian lake, several kilometers from the mouth of the nearest river. Evidently the rhythmic wave movement provides sufficient turbulences to substitute for the more typical water flow in river rapids.

Ameka (2000) studied the relationship between water chemistry and distribution of Podostemaceae in 21 rivers in Ghana. He concluded that the availability of appropriate substrata, in addition to adequate light, was more important than water chemistry in limiting the distribution of species. Quiroz et al., (1997) studied the relationship between the occurrence of Podostemaceae and water quality in 28 mexican rivers, 17 of which lacked detectable nutrient levels. Six rivers had detectable levels of total nitrogen (0.160–2.05 mgl^{-1}) and total phosphorus (0.10–0.72 mgl^{-1}). Contents of nutrients like Cl^-, Ca, Mg, NO_2, PO_4 and SO_4 are considerably low in the two study sites compared to Kerela rivers where the concentration of elements are as follows; SO_4: 20 mgl^{-1}; NO3: 16 mgl^{-1}; Mg: 1.6 mgl^{-1} to 4 mgl^{-1} (Mathew et al., 2003). These organic nutrients are formed primarily by biological processes and are contributed by the sewage of human activities. Philbrick & Novello (2004) reported that a population of some taxa (e.g. *Podostemum rutifolium*) appears to have been extirpated as a consequence of increased nutrient levels, which

indicates that the plants primarily occurred in low nutrient (oligotrophic) rivers. The most robust populations occurred in rivers with clean, nutrient-poor water. However, the plant may either need no nutrient or depend upon other sources for nutrient requirement in such a nutrient deficient condition. This aspect of ecology has to be taken care of in future research work.

In this investigation, all the three specimens viz., *Hydrobryum griffithii*, *Polypleurum wallichii* and *Podostemum subulatus* possesses chloroembryos, which implies that as soon as the embryo emerge out of the seed coat, it is capable of synthesizing its own photosynthetic product. It therefore plays an important role for the establishment and survival of the plant.

Toxic discharge of effluents from industries and agrochemical residues are serious threats to the existence of Podostemaceae that have a unique ecological requirement. Increasing impacts on tropical rivers are leading to loss of riverweed habitat, extirpation of populations and extinction of species. For instance, populations of temperate *Podostemum ceratophyllum* in New Hampshire were decimated after apparent industrial waste disposal in early 1970's (Philbrick and Crow 1983). In the Umtienger river, the construction of a new bridge as well as extraction of granite and coal causes considerable impact on the population of *Podostemum.subulatus*, where the size of the population has been reduced into almost 50 percent in 2004 when

compared to the observation made during the year 2003. Similarly, at present the erection of small bridges across the river Jainaw (near fossil park) will pose threat to the existence of species *Polypleurum wallichii* and *Hydrobryum grfthii*.

It is therefore imperative to evolve strategy for conservation concern in ex and in situ conditions. It is also reminiscent to relocate endangered species to alternative sites.

8.8 *Taxonomic considerations*

Phylogenetic relationship of aquatic angiosperms is difficult to reconcile because adaptation to hydric habitats is accompanied by extensive structural reduction and modifications that can obscure or even eliminate taxonomically useful characters (Les et al., 1997). This problem is exacerbated in the riverweeds whose lotic river rapid habitats have selected for highly unusual and extreme structural modifications. The systematic position of riverweed family remains enigmatic due to taxonomic difficulties imposed by the radically altered morphology of these alga-like angiosperms.

Although previous workers have placed this group phylogenetically among a wide variety of monocotyledons and dicotyledons, most contemporary authors have proposed that riverweeds are closely related to members of the dicotyledonous

order Rosales. A diversity of opinion also exists as to whether the Hydrostachyaceae are related to Podostemaceae.

As summarized in an early taxonomic history (Van Royen 1951), Podostemaceae were first described (Humbold et al, 1816) as a family related to monocotyledons (Alismataceae, Butomaceae, Juncaceae), a convention followed by many subsequent authors. Later opinions suggested affinities to other monocotyledons including Najadaceae (Schultz 1832), Lemnaceae and even Orchidaceae (Presl, 1830). Other unconventional associations were made with the families Ceratophyllaceae, Callitrichaceae and even the algal Characeae (Schultz 1832; Endlicher 1837, 1839). Lindley (1830), Schleiden (1839) and Gardner (1847) demonstrated the dicotyledonous embryo of Podostemacae. However, even with this interpretation the affinities suggested for the family (including Piperaceae, Nepenthaceae, Polygonaceae, Scrophulariaceae, Lentibulariaceae and Caryophyllacaee) remained vague (Van Royen 1951, Graham & Wood 1975).

The Podostemaceae have also been considered as close relatives of Hydrostachyaceae, because of various similarities and their unusual affinity for rheophytic habitats. However, Les et al., (1997) concluded that the rbcl sequences of Podostemaceae genera are substantially divergent from those of other angiosperms, including Hydrostachyaceae and other families believed to represent the closest extant sister group of river weeds.

Eventually embryological studies focused attention on Rosaceae, Saxifragaceae and Crassulaceae as possible relatives of Podostemaceae (Eichler 1886;Warming 1888; Willis 1902; Went 1910; Rombach1911). Arber (1920) recounted earlier opinions that Podostemaceae represented an ancient group related to Rosales and Sarraceniales and she suggested affinities to taxa such as Nepentheceae and Saxifragaceae. Subsequent authors (Hutchinson 1926; Engler 1930; Mauritzon 1933) continued to support the relationship of Podostemaceae to these families. Van Royen (1951) concluded that Saxifragaceae were essentially the sister group to a complex comprising Rosaceae, Crassulaceae and Podostemaceae, with the latter two families related most closely.

Takhtajan (1980) regarded the Podostemaceae as "related to and derived from" the order Saxifragales and followed by several authors (Mauritzon, 1933; Kapil 1970; Maheshwari 1945) who concluded that the family was derived from Crassulaceae.

Van Steenis (1981) also assumed that Podostemaceae were related to Rosales, but emphasized that further details of heir ancestry were obscure. The Rosalean alliance of Podostemaceae has not been accepted without reservation. Cronquist (1981) shared the opinion that Podostemaceae are related to either Saxifragaceae or Crassulaceae but still considered them to be 'taxonomically isolated'. Dahlgren (1980) placed Podostemaceae in super order Podostemiflorae, commenting that "...they are so specialized that

they cannot be associated with any other super order with out severe reservations". Corner (1976) argued that the seed structure of Podostemaceae was so different from Crassulaceae and Saxifragaceae "as to discredit the idea" of their relationship and believed that the group was more likely derived from Piperalean ancestors. Scogin (1992) compared the phytochemical profiles of Hydrostachyaceae and Podostemaceae, but found no evidence of close relationship between the families. Verdcourt (1986) suggested that evidence contrary to the relationship of Hydrostachyaceae and Podostemaceae had become "...too extensive to be argue against". An extreme view has been taken by Cusset & Cusset (1988b) who proposed the Podostemopsida as a class of angiosperms equivalent in rank to monocotyledons and dicotyledons.

Several classification of Posoatemaceae have been proposed (reviewed in Van Royen 1951). Taxonomists have treated the order Podostemales as comprising either the single family Podostemaceae Rich. Ex C. Agardh (Van Royen 1951; Cronquist 1981; Steenis 1981; Cook 1990). Van Royen (1951) proposed the recognotion of two subfamilies, Podostemoideae and Tristichoideae, each with two tribes. Willis (1914, 1926) and more recently, Cusset and Cusset (1988), Bremer et al. (1999) have proposed Tristichaceae as a segregate family. He placed both families in their "rosids" group associating them with such families as

Crossosomataceae, Krameriaceae, Staphyleaceae and Zygophyllaceae.

Most genera in the family are small (Cook 1990). Twenty-one (44%) are monotypic. Thirty five (73%) have five or fewer species, and only six have 10 or more species. In the new world, the largest genus is *Apinagia* (ca. 50 spp). About 70% of all the new world species are found in four genera: *Apinagia* (50 spp), *Marathrum* (25 spp), *Rhyncholacis* (25 spp), *Podostemum* (10 spp).

Despite the fact that, an unusual habit, morphology and other peculiarities of the natural history of Podostemaceae have attracted the attention of biologists since the early works of Gardner (1847), Willis (1902), Went (1929) and others, yet the taxonomic understanding of the family remains superficial till date. However, as taxonomic work progresses, the family is escalating rather than shrinking.

Chapter- 9

SUMMARY

- Two species *Hydrobryum griffithii* (Wall. Ex Griff.) Tul., and *Podostemum subulatus* Gardn. Have been rediscovered and re-described after Hooker (1885), from Meghalaya. The voucher specimens along with *Polypleurum wallichii* (R. Br. Ex Griff.) Warm have been deposited in the Eastern Circle, BSI, Shillong, India.
- The plants grow in rapidly flowing rivers and streams. And, are well adapted for the mechanical stress imposed by these harsh environments.
- The present study indicates that the Podostemaceae life cycles are linked to the hydrological cycle of their habitats, i.e., the high and low water periods. The population undergoes an annual cycle of colonization, establishment of a canopy of mature plant and die back when the water level drops.
- Till date, the previous workers mostly confined their studies on female gametophyte only, because of its remarkable reduced nature. The present investigation, apart from the megasporogenesis and embryogenesis, deals with the details of anther development and microspore formation in all the three members.

- The two stamens arise as an independent units (or primordia). Because of the intercalary meristematic activity in the andropodium, the stamen exhibit bifid structure in all the plants studied.
- Pollen grains are dispersed in pair, which is the characteristic feature of Podostemaceae. Pollen viability was assessed for the first time with *Hydrobryum griffithii* (Wall. Ex Griff.) Tul 73.68%, *Podostemum subulatus* Gardn., 36.17% and *Polypleurum wallichii* (R. Br. Ex Griff.) Warm 82.60%.
- Ovules are anatropous, bitegmic and tenuinucellate. Inner integument initiated after the differentiation of outer integument. In all the three genera studied, the hypodermal cell differentiated into archesporial initial, which inturn functions as megaspore mother cell.
- The development of embryo sac is bisporic in *Polypleurum wallichii*, while *Hydrobryum griffithii* and *Podostemum subulatus* follows monosporic development. Both *Dicraea* type and *Podostemum* type are encountered in *Polypleurum wallichii* against 5 nucleate, 4-celled structure Apinagia type B observed in Hydrobryum and Podostemum.
- Concomitant with the differentiation of embryo sac, the endothelium or integumentary tapetum is demarcated in these three plants. The integumentary tapetal cells are fully laddened with protein and starch grains; some of the cells become binucleate. This aspect has not been reported by previous workers.

- Nucellar plasmodium formation is initiated before fertilization in *Polypleurum wallichii*; *Hydrobryum griffithii* begins to develop nucellar plasmodium at the two-nucleate embryo sac stage. In *Podostemum subulatus*, it came into existence when the megaspore mother cell undergoes meiosis-I.
- All the previous workers conceived that all the members of Podstemaceae follow solanad type of embryogeny. However, an onagrad type of deveopment, for the revealed in *Hydrobryum griffithii* (Wall. Ex Griff.) Tul and; whereas *Podostemum subulatus* Gardn., *Polypleurum wallichii*(R. Br. Ex Griff.) Warm. shows solanad type.
- A preliminary survey on ecology of these three plants indicates that these plants occur in rivers with pH ranges from 6.84- 8.64; mostly grow in clean water with full sunlight and low nutrient condition.

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3	B Sc	NEHU	Botany	1997	II	59.25
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6	P hD	NEHU	Botany	Result awaited		

Research paper Published

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2. "Biodiversity of some members of Podostemaceae in Meghalaya and its potential use in paddy cultivation" In: K. Muthuchelian (ed), Ministry of Environment and Forests, Govt. of India, New Delhi, pp 91-94.

Research papers communicated:

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BIODIVERSITY OF SOME MEMBERS OF PODOSTEMACEAE IN MEGHALAYA AND ITS POTENTIAL USE IN PADDY CULTIVATION

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stract

Rivers, lakes and wetlands cover less than 1% of earth's surface, but their importance is immeasurable. Fresh ter environment harbor remarkable biodiversity and the aquatic species richness are of greatest importance in the pics. The members of Podostemaceae are popularly known as "river-weeds" and have markedly specialized and erse in their habits that are adapted to extreme habitats such as river-rapids and waterfalls. They harbor invertebrates, val fish, algae and likely important in tropical river ecosystem function. The present paper deals with the biodiversity hree species from Meghalaya, viz. *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus* Gard. and *lypleurum wallichii* (R. Br. ex Griff.)Warm., with illustrations for easy identification and of great phytogeographical erests.

Key words : *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus* Gard., *Polypleurum wallichii* Br. ex Griff.) Warm., Mycorrhiza, Cyanobacteria, Paddy cultivation, North East India.

TRODUCTION

The aquatic ecosystem and its biodiversity is an indication to gauge the value of a particular habitat. In India, rth-Eastern region and Western Ghats are the two world-renowned hot spots (Swaminathan, 1991; Mohan Ram Sehgal, 2001), which are of mega- biodiversity centre . These two regions are not completely explored. The nily Podostemaceae is the only representative of the Order Podostemales. It consists of aquatic angiosperms that ically grow on rocks in cascades, waterfalls and rapids where there are great fluctuations in the river water levels. e plants are cosmopolitan in tropical and warm regions, as extending into temperate North East America and East ia. They grow firmly attached to rocks and stones by means of adhesive rhizoid-like root system or haptera that rete mucilage. The vegetative plant body grow submerged during the rainy season, but are exposed to air when water level recedes, followed by flowering and setting fruit, dehydrating and eventually dying.

The pioneering work on Podostemaceae in India was carried out by Willis (1902 a, b). There are out 48 genera and 270 species worldwide; of which, about 11 genera and 42 species are reported in India (Cook, 96; Mohan Ram and Anita Seghal, 2001; Mathew, 2003); of which 21 species are endemic, largely confined to rela and Karnataka. The previous floristic studies of North East India (Haridasan & Rao, 1985; Joseph, 1983 and lakrishnan, 1983) did not mention anything about the existence of Podostemaceae members in Meghalaya state, lia. However, Kanjilal and Bor (1940) reported two species of Podostemaceae, which too, they referred from oker's Flora of British India (1885). The present paper deals with the biodiversity of the members from Meghalaya, *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus* Gard. and *Polypleurum wallichii* (R. Br. ex iff.)Warm., with illustrations for easy identification and of great phytogeographical interests. The voucher specimens ve been deposited at Botanical Survey of India, Eastern Circle, Shillong, India. Even though Mohan Ram & hgal (2001) reported that the members of Podostemaceae did not have any proven economic importance, the sent study deals with the potential usage of these plants for paddy cultivation especially in the hilly regions.

aterials and Methods

The following species of Podostemaceae viz. *Hydrobryum griffithii* (Wall. ex Griff.) Tul., *Podostemon subulatus*

Gard. and *Polypleurum wallichii* (R. Br. ex Griff.)Warm., were collected from Janiaw and Umtienger in Meghalaya state of North East India, (lying between latitude 25°. 02' and 26°. 07' N and longitude 89°. 49' and 92°. 50' E; with an elevation of about 2040m.above MSL). Both the vegetative and reproductive phases of these plants were observed in two consecutive years 2002 and 2003, giving the species description based on field observation. The associated organisms like Cyanobacteria and Mycorrhiza were located in the rhizoidal system as well as crevices of the plant body. The fixation of Nitrogen by Cyanobacteria was estimated by using gas-chromatography Hewlett Packard 4890 model. The mycorrhiza was identified as arbuscular and vesicular mycorrhiza. The photographs were taken by using Nikon E 600 microscope.

Boidiversity of Podostemaceae Members

The members of Podostemaceae are popularly known as "river-weeds" and have markedly specialized and diverse in their habits that are adapted to extreme habitats such as river-rapids and waterfalls. The members have unique morphological, anatomical and ecological features and stands clearly apart from all other angiospermous families. The biodiversity within this family is remarkable, resembling that of Algae and Bryophytes.

Hydrobryum griffithii

The plants are annuals, aquatics, growing on rocky surfaces in tropical streams, closely appressed to substrate and spreading over stones, more or less ovoid to circular outline. Frond coriaceous, green, lobed, patches about 10-15 cm wide, sending up buds clothed at the base with distichously scale-like imbricate leaves, enlarged at the base, tips caduceus. Leaves filiform- linear, greenish, scattered in groups of 2-3 on the upper surface of the thallus, up to 12 mm long. Flowers subsessile, zygomorphic, pedicellate, pedicels 2-6 mm long, remaining within the spathe. Stamens 2, borne on an andropodium, as long as the ovary, anther lobes golden yellowish, staminodes 2; Ovary subtriangular, green; stigma bifid, entire, wedge-shaped, brownish. Fruit capsule, isolobous, distinctly 12-ribbed. Seeds minute, numerous, elliptical-patelliform, surface granular.

Podostemum subulatus

Plants haptophyte, submerged minute herbs in rapid mountain streams, creeping and attached to rocks; thallus filiform, thread or ribbon like, frond lobulate, elongate, branched; buds on the edges of the lobes continuous with the veins; secondary shoots ascending. Leaves slender, subulate, very dense, obscuring the thallus when viewed from above. Flowers axillary, zygomorphic, naked, enclosed by tubular or funnel shaped spathe.

Stamens 2, with two transparent staminodes on either side of fertile stamen. Ovary ellipsoid, 2-locular, green, stigma bifid. Fruit capsule, unequally lobed, 8-10 ribbed, pedicelate, capsule valves rounded, persistent, incurved; seeds numerous, oval, minute.

Polypleurum wallichii

The plant body is a green or brownish flat, bilaterally symmetrical, fucoid thallus with wavy margins. It is fleshy, often branching exogenously and generally free drifting in the flowing water. It is attached at the base by a stout holdfast. Thallus various, usually freefloating with marginal ultimately 1-flowered secondary shoot.

Flowers zygomorphic, bisexual and hypogynous. Young flower bud is enclosed by a thin transparent membranous, sac- like spathe, staminal filament is forked, and each fork bearing four loculed anthers, light brown in colour. Ovary sessile, bicarpellary, syncarpous and elliptic, green with brown longitudinal ridges ; smooth when young, ripening into capsule, 8-ribbed; stigmas subulate, dark brown in colour. Fruit a capsule, isolobous.

DISCUSSION

The present report deals with the rediscovery of *Hydrobryum griffithii* (Wallich ex Griffith) Tul., *Podostemum subulatus* Gardner and *Polypleurum wallichii* (R. Br. ex Griff.)Warm. from Meghalaya State, India; that too after a long gap of 118 years. These species are belonging to the group Podostemoideae, which are highly specialized and occupy narrow aquatic ecological niches.

Ecological significances

Nowadays the aquatic biodiversity especially riverweed is important to conserve but they are not always treated with the respect they deserved (Cook, 1996). The ecological role of podostemads and biota associated with them in riverine system are largely unknown, especially when river rapids (Horne & Goldman, 1994) are highly productive.

The unique combination of characters presented by this family is unparallel among the angiosperms, leading to recent resurgence of world-wide interest. Riverweed habitats are under increasing pressures from reduced water quality and altering of water flow patterns (dam building). Increasing impacts on tropical rivers are leading to loss of riverweed habitat, extirpation of populations and extinction of species. The members are subjected to wide range of anthropogenic disturbances. Cross Bell (1990) have studied the effect of effluents of rubber factory in Kanyakumari District in Tamil Nadu, and observed the elimination of three species of Podostemaceae in the down stream due to acidic discharges. Predictions regarding global warming may also adversely affect Podostemads as they occur in seasonally pulsating rivers (Grimm 1993, Philbrick 1997).

The Podostemaceae are of great importance from agricultural point of view, because they depend upon other organisms such as mycorrhiza and cyanobacterial association for its nutrition. It can be used as biofertilisers for the improvement of yield in crop plants and increase the soil fertility particularly in paddy cultivation in the high altitude areas. The cyanobacteria show high nitrogenase activity (0.762 n mol C_2H_4 produced h^{-1}/mg^{-1} / FW). So, getting familiar with the ecology and biodiversity of the family will be of great help in –1) Conservation and management of species through behavioral ecology 2) Nutritional aspects of Podostemaceae and 3) increasing the yield of crop plants through incorporation of the Podostemaceae members.

As the plants are confined to the tropics, the light and temperature conditions are practically uniform. They are, however, subject to changes during the monsoons caused by the level and turbidity of the water. It is observed that flowers are produced even when the plants are submerged; perhaps, the clarity of the water and the intensity of light are more important factors. Among the three plants studied, there seems to be a certain degree of variation in the individual requirements of physiological maturity before flowering takes place, even in the same locality and under identical conditions. The biodiversity of Podostemaceae, thus offer challenging problems to the morphologists, the anatomists, the embryologists, the ecologists, and the physiologists through the problems associated with various aspects of their biology.

Use of podostemaceae for paddy cultivation

It is well established fact that vesicular and arbuscular endomycorrhia and cyanobacteria supplement the nutrition to the plants by supplying N,P and K. But so far, it has not been mentioned that these organisms are associated with members of podostemaceae. There are only three families in angiosperms which lack endosperm viz. Ordidaceae, Podostemaceae and Trapaceae. The present study reveals that the initial seed germination of Podostemaceae is associated with vesicular and arbuscular endomycorrhia similar to that of Orchidaceae. The family podostemaceae is divided into two sub families (1). Podostemoideae and (2).Tristichoideae, of which all the members of podostemoidaeae are haptophytes i.e. growing on rock surfaces but not penetrating into it. The members are autotrophic. How these plants meet their requirement N.P. and K? To get these three important elements, these plants depend on other organism. The associated organisms are cyanobacteria and vesicular and arbuscular endomycorrhia (see figure below), of which P and K are provided by VAM and N is supplied by cyanobacteria (nitrogenease produced 0.762 n mol C_2H_4 h^{-1}/mg^{-1} / FW). The plants can grow in the paddy field, because it can survive three to four months in the laboratory condition in a tray contain water (personal observations). By growing these plants it will enhance the soil fertility by supplying NP and K and thereby increase the productivity of paddy.

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