CHAPTER IV : REPRODUCTIVE BIOLOGY

Floral morphology and embryo sac development

P. stylosum var. *laciniata* (Wright) J. B. Hall

The flower is zygomorphic, lacks sepals, petals and bears only the stamens and pistil. Flower is covered by a spathella (Fig.9.B; Fig.26.B) along with two pairs of bracts (Fig.9.A; Fig.26.B) The pedicel measures ca. 2-3mm in young buds but it elongates ca. 14mm after pollination. The flower bud shows a laterally placed ovary and two stamens and their filaments are basally united into a thick, short filament forming a Y-shaped structure called andropodium (Fig.26.D). A pair of tepal, ca.2-3mm in length, arises at the base of the filament (Fig.26.D). The pistil consists of an egg-shaped ovary, a bipartite stigma but lacks a distinct style (Fig.26.C). The stigmatic lobes are short and lie close and bent over the ovary surface. The surface of the ovary has eight longitudinal ridges which become more prominent during fruit development and maturity. The anthers, when young, are pressed against the ovary wall and lie below the stigma (Fig.26.C). The filaments are ca. 1mm in young buds. At the time of pollination the filament elongate nearly four times of its length (3.5-4mm), curve inward and bring the anthers to the same level of the stigma (Fig.26.E). Further the andropodium elongates and the anthers extend beyond the stigma. The spathella which enclosed the flower bud ruptures laterally exposing the stigma and anthers within. After pollination the anthers are shed and the shrivelled andropodium persists at the base of the ovary.

Structure of Pollen, Pistil and Fruit

Pollen grains

SEM of pollen shows that they are dyads and there is some constriction at their point of union. The surface of the pollen shows echinate ornamentation (Fig.27.C). Pollen grain measures ca.36.56µm in polar diameter and an equatorial diameter of ca.21.10µm. The pollen number is ca.7000 in a flower (Table No. 10).

Transverse section of a young bud shows tetrasporangiate anther, two tepals, and stigma (Fig.28.A). The anthers are introrse, have four locules each and dehisce longitudinally. The mature anther wall is three cells thick (Fig.28.H). The endothecium shows wall thickenings (Fig.28.H). Two tepals emerged, one at each side of the andropodium base, are linear, have loose parenchymatous cells and lack vascular tissue (Fig.28.A, I). The pollen grains are released in pairs and are called dyads. It has an exine and intine layers (Fig.27.A).

Pistil

SEM of pistil show a long bifid stigma (Fig.27.E, F) which lie closely to the ovary wall and lacks a distinct style. The lobes of the stigma lie close to each other in young bud, the outer one covering the inner one. The two lobes start to straighten when the buds are exposed to air. Receptive time is usually in the morning.

The surface of the ovary looks zig-zag in nature (SEM) (Fig.27.E, F). About 356 ovules can be found per ovary (Table No. 10). The ovary is bicarpellary, syncarpous, bilocular, and ovules in an axile placentation (Fig.28.B). The ovary wall is six cells thick (Fig.28.F). The cells of outer epidermis and the three adjacent layers are large, thin-walled whereas the cells of the inner epidermis are thick and small (Fig.28.F). Ovarian septum is slender and formed by the epidermis of the carpels. The cross section of the stigma shows a transmitting tissue that takes up dense staining (Fig.28.E).

The ovules are anatropous, tenuinucellate and bitegmic. The inner integument is two cell layers thick and barely reaches the middle of the embryo sac. The outer is three cells layers thick (Fig.29.J). Both the integuments are rich in starch.

Pollen-Ovule ratio

The pollen:ovule ratio is found to be approximately 20:1 (Table No. 10).

Ovule-Seed ratio

The ovule:seed ratio is approximately 8:5 (Table No. 10).

Pollination type: This relatively low pollen:ovule ratio and high seed set suggests the possibility of self pollination. Moreover, the anthers are introrse and anthers lie towards the stigma and the flower is covered by spathella. These conditions also suggest the possibility of self pollination.

Fruit

The fruit is the capsule which is oval in shape, light brown in colour and measures 3.2 x 1.2mm. It has eight prominent longitudinal ridges along the wall. SEM of the fruit shows very prominent longitudinal ridges on the wall (Fig.27.I). Fruits dehiscence by two valves.

Seed

A fruit contain nearly 205 seeds arranged on an axile placenta. The seeds measures ca.266.38µm in length and ca.128.5µm in width (Table No. 10). The seeds are obovoid in shape.

Development of embryo sac

Megasporangium and nucellar plasmodium

In a transverse section of a young ovary, several ovular primordial arise from the placental lump (Fig.28.B). Ovule primordia first become apparent when the superficial cells are push up by sub-epidermal cells of the placenta. Subsequently, a column of 7-8 cells is surrounded by a unicellular layer, the so called nucellus (nucellar column and nucellar epidermis) expands and become curved (Fig.29.A). Then the external integument develops firstly from the base of the nucellus tissue and afterwards the internal (Fig.29.B). Both the integuments wrap the ovule which will reach its full curvature until it becomes anatropous.

The megaspore mother cell mgc occurs in the micropylar region of the nucellus (Fig.29.D). When meiosis starts, epidermis and central column of nucellar cells with dense cytoplasm below the megaspore mother cell enlarge and stretch, persisting in this way during all the meiotic process (Fig.29.D). The nucellar plasmodium completes its development by the time of embryo sac formation, as a consequence of the cell wall degradation of both the central column and lateral (epidermal) layers of nucellar cells (Fig.29.H).

Later, the integuments show a considerable increase in length (Fig.29.J) and the outer integument, composed of three cell layers forms the micropyle and contain numerous starch grains.

Megasporogenesis and embryo sac

Longitudinal section of mature ovary bears numerous anatropous, tenuinucellate and bitegmic ovules in a swollen axile placenta. The micropyle is formed by the outer integument only as the inner one lags behind the former. One of the megasporocyte (megaspore mother cell) differentiates in an ovular primordium (Fig.29.C). The megasporocyte is large with a conspicuous nucleus and dense, non-vacuolate cytoplasm (Fig29.C). The megaspore mother cell divides meiotically (Meiosis I) to produce two uninucleate dyads cells. The micropylar dyad cell degenerates and is recognized as a crescent-shaped cap at the micropylar end of the developing embryo (Fig.29.E).

The nucleus of the chalazal dyad cell divides meiotically (Meiosis II) to produce two megaspore nuclei of same size (Fig.29.F). These nuclei are not separated by a vacuole. Both the nuclei divide simultaneously to produce four free nuclei which are disposed in 1+1+2 manner (Fig.29.G). The organized female gametophyte has two small synergids at the chalazal end, a median egg and a polar cell at micropylar end (Fig.29.H.I) The synergids are usually juxtaposed and occasionally placed oblique. Sometimes, there is no wall between these two nuclei. Antipodal cells are absent.

The sequence of development of the embryo sac corresponds to the *Polypleurum* type. (Arekal and Nagendran, 1975b).

Floral morphology and embryo sac development

***Zeylanidium lichenoides* (S. kurz) Engler**

Flower buds are covered with a boat shaped, hard spathe and surrounded by three pairs of bracts (Fig.14.B; Fig.30.A,D). The spathe ruptures and exposes the flower during anthesis (Fig.30.D). From the base of the ovary, a long androphore bifurcate at the distal part and each bear a bilobed, dorsifixed anther. Two tepals are present on

either side of the andropodium and each measures a length of about 0.8-1mm (Fig.30.B). Filament is ca.1mm in the young bud but increase to three times at the time of pollination (Fig.30.E). Ovary is obovoid and smooth and has bifid stigma (Fig.30.E). During development and maturation the ovary wall develop eight longitudinal ridges. Fruit is obovoid, 8-ribbed capsule and measures about 1.5mm in length and 1mm in width. Each fruit contains about 45-50 seeds (Table No. 10). The seeds are very minute, dust like, ovoid and orange brown**.** Each seed measures about 203.19µm in length and 107.77µm in width (Table No. 10).

Structure of Pollen, Pistil and Fruit

Pollen grains

SEM studies show pollens are dyads and there is some depression at the point of contact. The surface of the pollen shows microechinate ornamentation (Fig.32.C). Nearly 4938 pollens dyads are produced from a single flower. It measures about 22.44µm polar diameter and an equatorial diameter of 14.67µm (Table No. 10).

T.S of anther shows that they are tetrasporangiate, (Fig.33.B) introrse and dorsifixed. Their wall is composed of three layers of cells (Fig.33.C). The endothecium shows wall thickenings. Pollen grains are released in pairs and called dyads. The pollen has two layers of exine and intine (Fig.32.B).

Pistil

SEM of pistil shows a bifid stigma which lie closely to the ovary wall (Fig.32.A,D). The short lobes of the stigma lie close to each other in young bud. The two lobes start to straighten when the buds are exposed to the air above the water. Receptive time is in the morning.

The surface of the ovary looks wavy in nature when examined under SEM (Fig.32.D). About 78 ovules are produced in a single ovary (Table No. 10). Transverse section of a young bud shows many young ovules in an axile placentation (Fig.33.A). Tepals emerged, one at each side of the andropodium base. They are linear and made up of loose parenchymatous cells and lack vascular tissue (Fig.33.D). The ovary is bicarpellary, syncarpous and bilocular.

Ovary wall is 6-8 cells thick. Cells of outer epidermis and two adjacent layers are large parenchymatous cells and inner epidermis are elongated and small (Fig.33.F). The inner epidermal cells are filled with some brown colour inclusions. Ovarian septum is slender and formed by the epidermis of the carpels. The ovary contains an apical septum. Placenta located at the middle of the septum and rich in starch grains.

Pollen-Ovule ratio

The pollen:ovule ratio is found to be nearly 63:1 (Table No. 10).

Ovule-Seed ratio

A fruit produces about 45-50 seeds and each seed measures about 203.19µm in length and 107.77µm in width. The ovule:seed ratio is found to be 8:5 (Table No. 10).

Pollination type: This relatively low pollen:ovule ratio and high seed set suggests the possibility of self pollination.

Fruit

The fruit is obovoid in shape, light brown in colour and measures 1.25 x 1mm. It has eight longitudinal ridges along the wall. SEM of the fruits shows the ridges along the wall (Fig. 32.I). Fruit dehiscence by two valves.

Seed

The fruit dehisces longitudinally when mature and seeds are dispersed. A fruit contain nearly ca.50 seeds arranged on an axile placenta (Table No. 10). The seeds are oval-elliptical in shape.

Development of embryo sac

Megasporangium and nucellar plasmodium

Several ovular primordial arise from the placental lump as seen in a transversely sectioned young ovary (Fig.33.A). A column of 5-6 cells is surrounded by a unicellular layer, the so called nucellus (nucellar column and nucellar epidermis) becomes expand and curved. (Fig.34.A). Then the outer integument develops firstly

from the base of the nucellus tissue and afterwards the internal (Fig.34.B). The ovule remains covered by both the integuments which will reach its full curvature until it becomes anatropous.

The megaspore mother cell (mgc) occurs in the micropylar region of the nucellus (Fig.34.C). When meiosis starts, epidermis and central column of nucellar cells with dense cytoplasm below the megaspore mother cell enlarge and stretch, persisting in this way during all the meiotic process (Fig.34.E). The nucellar plasmodium completes its development by the time of embryo sac formation due to the cell wall degradation of both the central column and lateral (epidermal) layers of nucellar cells (Fig.34.H).

Later, the integuments show a considerable increase in length (Fig.34.I) and the outer integument , composed of three cell layers forms the micropyle and contain numerous starch grains.

Megasporogenesis and embryo sac

Longitudinal section of mature ovary bears numerous anatropous, tenuinucellate and bitegmic ovules in an axile placenta. The micropyle is formed by the outer integument only as the inner one lags behind the former. One of the megasporocyte (megaspore mother cell) differentiates in an ovular primordium. The megasporocyte is large with a conspicuous nucleus and dense, non-vacuolate cytoplasm (Fig.34.C). The megaspore mother cell divides meiotically (Meiosis I) to produce two uninucleate dyads cells. The upper or micropylar dyad cell degenerates soon but persists as a dark cap over the developing embryo. (Fig.34.E). The dyad cell at the chalazal end undergoes the second meiotic division resulting in the formation of two nuclei. The nucleus at the micropylar end is larger than the nucleus at the chalazal end and the chalazal nucleus begins to show signs of degeneration (Fig.34.E). The micropylar megaspore nucleus after a free nuclear division produces two nuclei of approximately equal size (Fig.34.F). At this stage, the chalazal megaspore nucleus rarely persists. The two nuclei derived from the micropylar megaspore nucleus, in turn, divides and four free nuclei thus formed participate in the cellular organization of the embryo sac (Fig.34.G). The embryo sac consists of two synergids at the micropylar end, a central egg cell and a polar cell at the chalazal end.(Fig.34.H). There are no antipodal cells.

The sequence of the development of the embryo sac corresponds to the *Apinagia* type B (Battaglia, 1987; Nagendran et at., 1977).

Floral morphology and embryo sac development

***Willisia selaginoides* (Bedd.) Warming ex. Willis**

The flower is zygomorphic, lacks sepals, petals and bears only the essential organs. The flower bud is covered by a spathella whose tip has two projections. The flower bud shows a laterally placed ovary and two stamens which are basally united into a thick, short filament forming a Y-shaped structure called the andropodium (Fig.35.E). A pair of tepal, ca.5mm in length, arises at the base of the filament. The pistil consists of an elliptical-shaped ovary, a bifid stigma but lacks a distinct style (Fig.35.B). The stigmatic lobes lie close and bent over the ovary surface. The surface of the capsule has four longitudinal ribs along the wall (Fig.35.J). The anthers, when young, are pressed against the ovary wall and lie below the stigma. The filaments are ca. 1.5mm in young buds. At the time of pollination the filament elongate nearly 6-8mm, curve inward and bring the anther to the same level of the stigma. Further the andropodium elongates and the anthers extend beyond the stigma. The spathe which enclosed the flower bud ruptures laterally exposing the stigma and anthers within. After pollination the anthers are shed and the shrivelled andropodium persists at the base of the ovary.

Structure of Pollen, Pistil and Fruit

Pollen grains

Pollens are dyads and there is depression at the point of contact. The surface of the pollen shows microechinate ornamentation (Fig.35.H). A single flower produces nearly 8135 pollens grains. It measures about 29.04µm polar diameter and an equatorial diameter of 19.35µm (Table No. 10).

Anthers are tetrasporangiate, introrse, dorsifixed. The wall of the mature anther is composed of three layers of cells. The endothecium shows wall thickenings. Pollen

grains are released in pairs and called dyads. It has two layers exine and intine (Fig.35.G).

Pistil

SEM of pistil shows a bifid stigma that curved towards the ovary surface. The two lobes of the stigma lie close to each other in young bud. The two lobes start to straighten when the buds are exposed to air. Receptive time is in the morning before noon.

The surface of the ovary looks like a bee-hive with many polygonal cells when viewed under the SEM (Fig.35.F). About 252 ovules are produced per ovary (Table No. 10). Transverse section of a young bud shows tetrasporangiate anther and ovules in an axile placenta. Spathella is non-vascularised lamina and consists of 3-4 layers of cells. Tepals emerged, one at each side of the andropodium base. They are linear and made up of loose parenchymatous cells and lack vascular tissue (Fig.37.E). Longitudinal section of the pistil shows that the stigma lobes are unequal (Fig.37.B). Ovary is bicarpellary, syncarpous, bilocular and ovules in an axile placentation. Ovary wall is eight cells thick. Cells of the inner epidermis are small and thick walled. The inner epidermis and adjacent cells are filled with some dark brown substances (Fig.36.F) which is mainly carbohydrate. The layer next to it is narrow and radially elongated cells. The outer epidermis and the two subjacent layers consist of large, thin-walled cells which contain starch granules. Placenta is located at the middle of the septum and rich in starch grains.

Fruit

The fruit is oval-elliptical in shape and measures ca. 4mm length and width of ca.3mm. SEM of the fruit shows two slightly prominent longitudinal ribs along the wall of one side of the fruit (Fig.35.J). Fruit dehiscence occurs by two valves.

Seed

A single fruit produces about 184 seeds and each seed measures about 274µm in length and 206µm in width (Table No. 10). The seed are ovoid in shape.

Pollen-Ovule ratio

The pollen:ovule ratio is found to be 32:1 (Table No. 10).

Ovule-Seed ratio

A fruit produces about 184 seeds and an ovary produces about 252 ovules so the ovule:seed ratio is found to be 6:5 (Table No. 10).

Pollination type: This relatively low pollen:ovule ratio and high seed set suggests the possibility of self pollination.

Development of Embryo sac

Megasporangium and nucellar plasmodium

The megaspore mother cell (mgc) occurs in the micropylar region of the nucellus (Fig.38.A). When meiosis starts, epidermis and central column of nucellar cells (nc) with dense cytoplasm below the megaspore mother cell enlarge and stretch, persisting in this way during all the meiotic process (Fig.38.B). The nucellar plasmodium completes its development by the time of embryo sac formation due to the cell wall degradation of both the central column and lateral (epidermal) layers of nucellar cells (Fig.38.F).

Later, the integuments show a considerable increase in length (Fig.38.G) and the outer integument , composed of three cell layers forms the micropyle and contain numerous starch grains.

Megasporogenesis and embryo sac

Longitudinal section of mature ovary bears numerous anatropous, tenuinucellate and bitegmic ovules in an axile placenta. The micropyle is formed by the outer integument only as the inner one lags behind the former. One of the megasporocyte (megaspore mother cell) differentiates in an ovular primordium. The megasporocyte is large with a conspicuous nucleus and dense, non-vacuolate cytoplasm (Fig.38.A). The megaspore mother cell divides meiotically (Meiosis I) to produce two uninucleate

dyads cells (Fig.38.C). The upper or micropylar dyad cell degenerates soon but persists as a dark cap over the developing embryo (Fig.38.D). The dyad cell at the chalazal end undergoes the second meiotic division resulting in the formation of two nuclei (Fig.38E, Diagrammatic). These two nuclei divide resulting in formation of four nuclei that participate in the formation of the embryo sac. The embryo sac consists of two synergids at the micropylar, an egg cell and a polar cell at the chalazal end (Fig.38.F).

The sequence of the development of the embryo sac corresponds to the *Podostemum* type (Battaglia, 1971).

Table No. 10. Comparison of reproductive structures of *P. stylosum* var *laciniata*, *W. selaginoides* and *Z. lichenoides*

Parameters	***P. stylosum* var *laciniata***	***W. selaginoides***	***Z. lichenoides***
Fruit size Length(mm) Width (mm)	 3.21 1.26	 3.7 1.5	 1.5 1
Number of ribs on fruit	8	4	8
Seed size Length(µm) Width(µm)	 266.38 128.5	 274.5 206.6	 203.19 107.77
Number of seeds per fruit	205.9	184	45
Total no. of pollen dyad per flower	6958	8135	4938
Size of pollen dyad Polar diameter(µm) Equatorial diameter (µm)	 30.56 21.1	 29.04 19.35	 22.44 14.67
Number of ovules per ovary	356	252	78
Pollen ovule ratio	20:1	32:1	63:1
Ovule seed ratio	8:5	6:5	8:5
Length of pedicel in the mature fruit (mm)	14.0	16.0	4.0

DISCUSSION

In the subfamily Podostemoideae, each floral bud is protected by spathella, a unique and characteristic structure, generally of uniform thickness. It is composed of epidermis and 2-3(-5) layers of parenchyma (Jäger-Zürn, 2005). In the present study, *P. stylosum* var. *laciniata* shows 3 layers including an epidermis, *Z. lichenoides* and *W. selaginoides* show 3 layers of parenchyma and a single layer of epidermis. The growth of the bud and its frequent upright position, coupled with the pedicel elongation, leads to spathella rupture at anthesis. The nature of the spathella, however, has been the subject of discussion. At present, the spathella is generally seen as established of one or more hypsophylls, even though no vascular tissue or midrib indicates leaf features. It is considered as an arillus (Jäger-Zürn, 2005) possibly because of the small and coriaceous fruit similar to a seed. In *Marathrum foeniculaceum* and *M. utile* the occurrence of two tips in the young spathella supports the view that the spathella is formed from two fused bracts. Willis (1902a) interpreted the spathella as a combination of one or two entire leaves. The occurrence of two tips in spathella is also found in the spathella of *W. Selaginoides.* It is noteworthy that the decussate position of the tips is perpendicular to the distichous foliation of the leaves (Jäger-Zürn, 2005). The shoots of *Diamantina lombardii* Novelo, Philbrick and Irgang, a Brazilian podostemoid, produce two-flowered units, each one with a terminal flower surrounded by a tubular spathella and a lateral flower subtended by an open bract-like spathella (Rutishauser et al., 2005). This genus seems to be one of the most basal in the New World Podostemoids (Ruhfel et al., 2011).So the open bract-like spathella can be derived from a foliage leaf and should be taken as a plesiomorphic character state within the subfamily Podostemoideae. In other clusioids, for example the family Clusiaceae, bracts are often in close contact with the perianth parts and do not differ very much from them in form and size.

Floral reduction in Podostemaceae is accompanied by the development of a wide range of vegetative structures. These vegetative parts die quickly when exposed to dry conditions. Arber (1920) stated " there is little doubt that podostemads are derived from some terrestrial group since the structure of flowers are usually fragrant in the least specialized genera such as *Indotristicha* and are insect pollinated. They possess a perianth of three, free or connate, bract-like segments with three filiform styles. In

Polypleurum, Podostemum, Farmeria, Zeylanidium, Willisia the number of stigmas is concomitantly reduced to two. In the present study the pollen grains of all the three species remain dyads, being ca. 36.56µm in length and ca. 21.10µm width in *P. stylosum var laciniata*, 22.44µm in length and 14.67µm width in *Z. lichenoides* and 29.04µm length and 19.04µm width in *W. selaginoides*. Philbrick (1984) recorded a dyad length of ca. 39µm and a width of 23µm in *Podostemum ceratophyllum.*

The morphology of pollen in *Polypleurum, Zeylanidium* and *Willisia* is typical of the Podostemoideae. The grains have an echinate surface. Such pollen grains have also been reported in *Griffithella hookeriana* by Vartak and Kumbhojkar (1989) and by Vidyashankari and Mohan Ram (1987). Pollen dyads are also reported to occur in Scheuchzeriaceae and some Annonaceae (Char and Nagendran, 1974; Dahlgren and Clifford, 1982; Pacini et al., 1985). Spinulose pollen grains are also found in various Podostemoideae e.g. *Mourera* and *Marathrum* (Novelo and Philbrick, 1993; O Neil et al., 1997).

The pedicel elongates considerably in all three presently studied plants studied here and raises the flower at the time of pollination. Van Royen (1951) noted the doubling of pedicel length due to post-anthesis growth in various *Rhyncholacis* spp. and other Neotropical Podostemoideae.

Seed production is generally prolific in Podostemaceae. Capsule dehiscence leads to the release of tiny (<0.5 mm. long), dry seeds. Philbrick and Novelo (1997) reported the production of 14,000 and 70,000 seeds per 5 cm^2 area for the Mexican species *Oserya Coulteriana* Tul. and *Marathrum rubrum* Novelo and C.T. Philbrick, respectively. Grubert (1974) documented seed production in per plant may range from 2360 for to 1,020,000. Between 2000 and 2400 seeds per fruit have been counted in *Mourera fluviatilis* (Rutishauser and Grubert, 1994), nearly 590 seeds in *Apinagia multibranchiata* and approximately 720 seeds in *Rhyncolacis penicillata* (Grubert, 1974) and 278±52 in *Cladopus hookerianus* (Vidyashankari, 1988a). In the present study ca.205 seeds are found in *P. stylosum* var. *laciniata*, ca. 50 seeds are produced in *Z. lichenoides* and ca. 108 seeds are counted in a fruit of in *W. selaginoides*. Seed production in both annual and perennial Podostemaceae is notable because it contrasts with the general pattern among aquatic angiosperms for reduction in flowering and seed production (Sculpthorpe, 1967; Barrett et al., 1993; Les and Philbrick, 1993;

Philbrick and Les, 1996). These examples illustrate that podostemads typically have high reproductive potential.

The number of ovules borne in an ovary varies among the species of Podostemaceae. Philbrick and Novelo (1997) reported that the ovule number varies from 35 in *Podostemum ricciiforme* to 996 in *Marathrum rubrum.* The ovule number in *P. stylosum* var *laciniata* is found to be ca. 356, ca. 78 in *Z. lichenoides* and ca. 252 in *W. selaginoides*. Philbrick and Novelo (1997) found that there is a significant difference between ovule number for annual species and perennial species. Annual species produce significantly greater ovule number than perennial species and the three plants studied here are all annual species. This corresponds to the general pattern observed in other angiosperms (Richards, 1986). The riverweeds produce seed in abundance and seed set may even reach tens of millions per population (Philbrick and Novelo, 1994).

The characters of the fruit are of taxonomic importance within the Podostemaceae (Nagendran, 1975). Like other Indian Podostemaceae, the fruits of presently studied three species are dehiscent. The ovary is smooth but ribs appear when the capsule matures. Eight longitudinal ribs are present in *P. stylosum var laciniata* and *Z. lichenoides* and four in *W. selaginoides.* When flowers are exposed to air after fertilization, they quickly mature into fruits. Rapid maturation of fruits appears to be a characteristic feature of podostemads (Mohan Ram and Sehgal, 1997). In general, aquatic angiosperms produce only a moderate number of seeds (Sculthorpe 1967). Philbrick and Novelo (1997) stated that the podostemads with annual life cycle produce far more seeds per fruit than those perennials. Willis (1902a) commented that the fruits and seeds of podostemads are unsuited to shed in fast flowing water where they have little chance to germinate. Production of a large number of seeds appears to be an adaptive device in an environment in which the chances of survival and dispersal are meagre. Podostemads do not produce asexual propagules and vegetative growth occurs only from the existing plants. This could be the reason that the population is restricted to a limited area.

The apical septum is a rare morphological peculiarity occurring in bicarpellate ovaries which combine into a short style (Hartl, 1962). Although the apical septum is otherwise infrequent among angiosperms, it is quite common in Podostemaceae

(Jäger-Zürn, 1997a, 2003). The presence of the apical septum has been incidentally observed among Podostemoideae. The occurrence of apical septum in the Podostemaceae may be informative with regard to an evolutionary pathway.

Cell bodies and cell inclusions of unidentified nature are present in *Polypleurum*, *Zeylanidium* and *Willisia*. Such features of unknown chemistry are also reported among species of Podostemoideae. Additionally, long secretory cells occur in neotropic members of the subfamily (Jäger-Zürn et al., 2007). These features may be promising in traits finding attention in phylogenetics analyses.

Pollen-ovule ratio and ovule-seed ratio determine the breeding systems. Pollen-ovule ratios of flowering plants are generally indicative of the chances of pollen reaching the stigma, ensuring the maximum seed set and thus determining their breeding systems (Cruden, 1977). Okada and Kato (2002) have inferred pollination systems from pollen-ovule ratio in 20 species of Podostemaceae. They found low pollen-ovule ratio in autogamous podostemads while allogamous species have high values. In the present study, pollen-ovule ratio is found to be low which suggests that the species are autogamous.

Autogamy ensures maximum seed production. This is a significant observation as asexual reproduction has not been reported in Podostemaceae (Philbrick, 1997). The podostemads unlike other aquatic angiosperms are prolific seed producers probably necessitated by wastage by air/water currents. Thus, podostemads, in general, represent an example in which all reproductive energy seems to have been channelized into high seed production, replacing asexuality with sexuality.

Development of embryo sac

In the present study, two of the three embryological characteristics that consistently distinguish podostemaceae from other angiosperms are observed: (1) 4-nucleate, 4-celled mature embryo sac; (2) the occurrence of a nucellar plasmodium. The third trait, the absence of double fertilization and endosperm formation (Haig, 1990; Raghavan, 2003), although not confirmed, is likely to occur.

Most embryological studies in Podostemaceae are based on Indian taxa that represent subfamily Podostemoideae.

The gynoecium is bicarpellary, syncarpous and bilocular in all the three species presently studied. A superior ovary bearing numerous minute ovules on a massive axile placenta is bilocular in Podostemoideae (Arekal and Nagendran, 1977b) of the Podostemaceae. Therefore, the reports of the ovary being unilocular with free central placentation (Rendle, 1925; Ramamurthy and Joseph, 1964) are not confirmed and are erroneous records for the family.

The ovular primordial arises on the placental hump as protuberances and a hypodermal cell differentiates early in the ovule which bends in the form of a hook, causing its distal region to move closer to the placenta. Differentiation of the two integuments is simultaneous; but the outer one grows faster than the inner which lags behind. Thus, only the outer integument organizes the micropyle.

The differentiation of anatropous, bitegmic and tenuinucellate ovules from ovular primordial is almost uniform in the family. Later, well-defined and related patterns in the development and organization of the female gametophyte in different taxa of Podostemaceae follow. Conventionally, they are classified as the *Apinagia* type, the *Polypleurum* type and the *Podostemum* type (Battaglia, 1971). Each of these ontogenies commences as a densely cytoplasmic large nucleated hypodermal cell which differentiates early in the nucellus. It enlarges and directly transforms into a megaspore mother cell and remains at this stage until the level of water subsides in the streams in which these plants grow (Nagendran et al., 1977), exposing mostly the reproductive parts.

The megaspore mother cell undergoes asymmetric first meiotic divisions. Of the resultant dyad cells, the micropylar dyad cell degenerates, without further division and is usually seen as an inverted cap of dark mass at the top of developing female gametophyte. It has no functional participation in the subsequent development and organization of the female gametophyte. On the contrary, the chalazal dyad cell is distinctively large with a prominent dense protoplasmic mass, functional in the second meiotic division of the ontogeny. The phenomenon of unequal first meiotic division is reported in other investigated taxa of Podostemaceae (Arekal and Nagendran,

1975a, b,1976, 1977b) Nagendran and Arekal, 1976; Nagendran et al., 1976, 1977, 1980), *Epipogium roseum* (Arekal and Karanth, 1981).

In *Zeylanidium lichenoides,* the development of the embryo sac corresponds to the *Apinagia* type (Nagendran, 1974). The "*Apinagia* type" of embryo sac brings about two synergids at the micropylar side, the egg cell and a single polar cell in the chalazal side of the embryo sac. Interestingly, the single polar cell has no apparent function as double fertilization does not occur (Battaglia, 1987). Antipodal cells are absent.

The "*Apinagia* type" of embryo sac development in Podostemaceae was first described by Went (1908, 1910, 1912, 1926a). It was considered a reduced "*Allium* type" (Maheshwari, 1947). The "*Allium* type" is one of the main categories of embryo sac development in angiosperms (Swamy and Krishnamurthy, 1975). Battaglia (1971), however, had emphasized that "the mature embryo sac of Podostemaceae is unique in its organization" and question whether the "reduced *Allium* type" designation was appropriate.

In *Z. lichenoides*, the micropylar dyad cell degenerates and persists only as a crescent-shaped cell. The nucleus of chalazal cell of the dyad completes meiosis – II producing two free nuclei. The chalazal nucleus of the two-nucleate embryo sac degenerates and only the micropylar nucleus is functional in the final embryo sac structure. According to Maheshwari (1937) and Nagendran (1974), the type of embryo sac in *Z. lichenoides* is monosporic in origin since only the micropylar nucleus in the chalazal dyad cell is functional in embryo sac formation. This micropylar nucleus in the chalazal dyad cell divides twice and produces four nuclei (or four uninucleate cells). From the biological point of view, the chalazal nucleus does not contribute to the subsequent formation of the gametophyte (Maheshwari, 1955; Arekal and Nagendran, 1975a) and henceforth does not contribute to the genetic variability of the embryo sac.

The nucleus of the chalazal dyad cell completes the second meiotic division to produce two megaspore nuclei without cell wall formation between them. These nuclei are separated towards opposite poles of the female gametophyte by a vacuole. The report of cell wall formation at this two-nucleate megagametophyte stage in *Polypleurum elongatum* (Magnus, 1913) and *Griffithella hookeriana* (Razi, 1949)

are not confirmed in the present study. This corresponds to the observations for *Polypleurum stylosum* (Mukkada, 1964), *P. dichotomum*, *P. filifolium* and *P. munnarense* (Nagendran et al., 1977). Until this primary two-nucleate phase, the female gametophyte development is specific in nature and fairly uniform in the family. This stage of megasporogenesis can be considered to be characterized with the normal number of meiotic divisions (two) but reduced and censored sporophytic genetic material. The other genetic component continues to disintegrate without functional role in the subsequent gametophyte ontogeny. The latter process means less utilization and considerable preservation of the maternal food resources. This is because of the reduced quantity and greater quality of the meiotic products in the course of the megasporophytic phase. Thus, megagametophytic phase ensue a censored genetic material (functional) and enough energy supply (preserved maternal metabolic substances-food resource) from the previous two stages of the ontogeny. This, itself shows an evolutionary process, exemplified by a high degree of reductionalism during the megasporophytic phase. This contention is an established fact in the various investigations because highly advanced megagametophytic ontogenies over a spell of time have evolved as progenies of the primitive - *Polygonum* type in diverse and advanced families of the angiosperms.

The ontogenies must be the result of several factors: polarity, course of nutrient supply and other aquatic factors that come into interplay during a given ontogeny. Concurrently, either the megasporophytic or megagametophytic or in both phases, part of the whole or even the whole corresponding genetic material is made to be functional. This is natural mechanism for the existence and survival of a given gametophyte ontogeny during adverse conditions and habitats, among the competing taxa. Thus, the theory of megaspore conflict of Haig (1986) suggests that the somatic spores or their derivatives having a role in successful gametophyte function have a tendency to become non-functional. This may ascertain the strike phenomenon of Davis (1966), reported in Podostemaceae (Nagendran et al., 1977, 1980) and confirmed to be taking place in the post-meiotic mitosis in the present study. This is exhibited by the total disintegration of the primary chalazal nucleus which commences after the cell wall of the nucellar cells disorganize, below the female gametophyte. This nucleus completely disappears as soon as a distinct vacuole separating it from the micropylar megaspore one becomes invisible. Similar

phenomenon does occur soon prior to the cellularization of the megagametophyte starts for the investigated taxa of Tristichoideae (Arekal and Nagendran, 1976). This strike phenomenon has been reported in *Epipogium roseum* (Arekal and Karanth, 1981); but it is partially or complete, in similar biological specialized family, Orchidaceae (Abe, 1972).

Nagendran (1974) reinterpreted *Apinagia* type as monosporic and unreduced bisporic embryo sac development as earlier embryologists viewed. This classification corroborates the present observations that only the micropylar megaspore nucleus contributes all the four nuclei which alone participate in the cellular organization of the mature female gametophyte. This concurs with the criterion explicitly expressed in the original definition of classifying female gametophyte in the angiosperms by Maheshwari (1937) and pointed out by Nagendran (1974) that an embryo sac formed from the divisions of a single megaspore nucleus should be called monosporic; and when the two take part in its development, it is bisporic; and when all the four contribute to it, it is tetrasporic. Due to the strike phenomenon the chalazal megaspore nucleus is eliminated in the ontogeny. This is also reported in other investigated taxa of Podostemaceae (Mukkada, 1969; Arekal and Nagendran, 1977b; Nagendran et al., 1977). It is prudent that, the number of megaspore nuclei at the prime stage of the megagametophyte formation, its nuclear mitotic products and their subsequent participation in the cellular organization of mature female gametophyte needs to be considered while classifying its types in the angiosperms. The *Apinagia* type is justified to be reinterpreted as monosporic (Nagendran, 1974) because only one megaspore nucleus undergoes the first and second nuclear mitotic divisions producing all the four nuclei that alone participate in the cellularization and are present in an organized female gametophyte.

Sehgal et al., (2010) tracked the stages of megasporogenesis and megagametogenesis in *Dalzellia zeylanica* (Tristichoideae) and concluded that although the ontogenic pattern of the female gametophyte in *D. Zeylanica* parallels that shown by earlier workers (Battaglia, 1971; Razi 1949, 1955; Arekal and Nagendran 1977b; Mukkada, 1969) up to the stage of differentiation of the egg apparatus and central cell, its subsequent development differs. The single polar nucleus of the central cell in *D. Zeylanica* degenerates soon after its formation, resulting in unique, highly reduced,

three nucleate, three-celled mature female gametophyte consisting only of the egg apparatus (Sehgal et al, 2010). The degeneration of the 'polar cell' has also been reported in *Tristicha trifaria* (Tristichoideae; Sikolia and Ochora, 2008). Although the evolutionary significance of this type of female gametophyte is not yet clear, it certainly extends the reported variability in the developmental patterns of the female gametophyte in angiosperms.

In *Polypleurum stylosum* var. *laciniata* the development of the embryo sac corresponds to the "*Polypleurum* type". Magnus (1913) reported that the first division of the functional dyad nucleus in *Polypleurum elongatum* is followed by the formation of a wall resulting in a two-celled embryo sac after which the upper cell divides transversely to give rise to a synergid and an egg, while the lower cell divides anticlinally to form the two antipodal cells. Thus, according to Magnus (1913), there are no free nuclear divisions at all which would be a unique case among angiosperms. Mukkada (1962a, 1962b, 1964) after investigating *P. elongatum* and *P. stylosum* confirmed the presence of a four-celled embryo sac reported by Magnus but observed no wall formation after the first division of the functional dyad nucleus. Razi (1966), after investigation of *P. agharkarii*, agreed with Mukkada's observations. Nagendran et al., (1977) confirms the account given by Mukkada (1962a, 1962b, 1964) and Razi (1966) regarding the sequence of the development of the embryo sac in *P. dichotomum* and *P. munnarense*. The present study on development of embryo sac of *P. Stylosum* var *laciniata* confirms the account given by Mukkada (1962a, 1962b, 1964), Razi (1966) and Nagendran et al., (1977) in *P. dichotomum* and *P. munnarense*.

Nagendran et al., (1977) did not agree with the interpretation of Magnus (1913), Mukkada (1962a, 1962b, 1964), Razi (1966) and Battaglia (1971) that the "*Polypleurum* type" of embryo sac consists of one synergid, one egg- both considered as sister cells and two antipodal cells. In Podostemaceae, there is an unmistakable trend towards the elimination of nuclei at the antipodal and of the embryo sac. This is quite well seen in the *Apinagia* type of embryo sac where the primary chalazal nucleus which is separated from the primary micropylar nucleus by a vacuole at the two-nucleate embryo sac stage, starts degenerating from the time of its appearance. In the *Polypleurum* type of embryo sac, the absence of a vacuole at the two-nucleate

stage should be considered as an important clue for the complete elimination of the primary chalazal nucleus. Therefore, the two nuclei of the two-nucleate embryo sac of the *Polypleurum* type after a mitotic division produce the micropylar quartet of nuclei. Nagendran et. al. (1977) reported that there are no antipodal cells as stated by Magnus (1913), Mukkada (1964), Razi (1966) and Battaglia (1971). The micropylar quartet of nuclei can only organize into two synergids, an egg and a polar cell. As stated by Battaglia (1971), the free nuclei of the *Polypleurum* type of embryo sac attain their cellular morphology according to their axial position along the embryo sac. Therefore, during organization the embryo sac attains reverse polarity i.e., the synergids occupy the chalazal pole and the single polar cell takes its position at the micropylar pole of the ovule. The findings of the present study correspond to the findings of Nagendran et al. (1977).

Magnus (1913) and Chiarugi (1933) (studying *Podostemum subulatum* Gard. and *Farmeria metzgeriodes* (Trimen) Willis, and *Weddellina squamulosa*, respectively) reported another type of embryo sac development termed the '*Podostemum* type'. This embryo sac type shows a true bisporic origin. Only one mitotic division takes place (instead of two in the '*Apinagia* type') to form the micropylar quartet. The two horizontally arranged synergids arise from the upper megasporial nucleus. In the present study the development of embryo sac in *Willisia selaginoides* follows the *Podostemum* type same as reported by Arekal and Nagendran, (1976). The arrangement of nuclei is T-shaped with the two synergids, one egg cell and one polar cell in the embryo sac. As in the '*Apinagia* form', the polar nucleus has no apparent function. Maheshwari (1947, 1955), and Battaglia (1971) later doubted the interpretation of Magnus (1913) and Chiarugi (1933) and pleaded for a reinvestigation.

The '*Polypleurum* type' differs from the '*Podostemum* type' in the arrangement of the spindles of the only mitotic division of the two megaspores with a vertically arranged upper spindle and horizontally arranged lower spindle. This results in only one synergid and an egg cell (from the upper megaspore) and two horizontally arranged cells (from the lower megaspore) at the chalazal side of the embryo sac.

In *Hydrobryopsis sessilis* (Willis) Engl. not only the '*Polypleurum* type', but also the questioned '*Podostemum* type', were found simultaneously (Arekal and Nagendran,

1975b). But Battaglia (1987) continued to question the occurrence of all bisporic embryo sacs in Podostemaceae and strongly encouraged a reinvestigation of both the '*Polypleurum* type' in species of *Polypleurum*, *Hydrobryopsis sessilis*, *Willisia selaginoides* and *Zeylanidium johnsonii*, and the '*Podostemum* type' in *Hydrobryopsis sessilis* and *Weddellina squamulosa*. It is notable that in a subsequent study, Nagendran et al., (1980) described the five-nucleate monosporic "*Apinagia* type' instead of the previously reported '*Podostemum* type' in their reinvestigation of *Podostemum subulatus.*

Existence of the *Podostemum* type in the family was questioned time and again (Maheshwari, 1937, 1941, 1947; S. C. Maheshwari 1955, Battaglia, 1971, Arekal and Nagendran, 1975a). Nevertheless, Arekal and Nagendran, (1975b, 1976) proved the existence of the *Podostemum* type in *Hydrobryopsis sessilis* and *Willisia selaginoides* (Bedd.) Warm. ex Willis. In this type also, there is complete suppression of the formation of the chalazal megaspore nucleus which regularly degenerates in the *Apinagia* type of embryo sac. The four nuclei that are produced prior to the organization of the sac belong to the micropylar quartet only. The components of the organized *Podostemum* type of embryo sac are two synergids at the micropylar end, an egg and a haploid polar cell.

The degeneration of the polar cell before fertilization, leading to the absence of double fertilization, is characteristic for Podostemaceae. The reason for this suppression is unclear and challenging, and probably involves failure of the pollen tube to discharge the second gamete (Raghavan, 2003; Sikolia and Ochora, 2008; Sikolia and Onyango, 2009; Sehgal et. al., 2010).

Pseudo-embryo sac

A feature of the Podostemaceae, unmatched in any other angiosperm family, is the development of the pseudo-embryo sac. According to Hammond (1937), the cells of the nucellar near base of the inner integument fail to divide rapidly enough to keep pace with the elongation of the integuments, and these cells are consequently drawn out into shredded structures, later giving a cavity, the ‘pseudo-embryo sac,’ surrounded by the inner integument, and into which, the developing embryo grows.

The nutritional function is performed by this nucellar plasmodium (Cook and Rutishauser, 2007)

The pseudo-embryo sac is a characteristic feature of all the Podostemaceae so far investigated. In Podostemaceae, there are two fundamental patterns of development of the pseudo-embryo sac/nucellar plasmodium. In subfamilies Podostemoideae and Weddellinoideae, the nucellar plasmodium begins to develop prior to fertilization, whereas in subfamily Tristichoideae, this structure develops only after fertilization (Nagendran et al., 1977). However, in both patterns the disintegration of the cell walls between the nucellar cells leads to the fashion of uninucleate protoplasts that has been referred as a "pseudo embryo sac" by Went (1910), Magnus (1913) and others. 'Nucellar plasmodium' is more appropriate term to designate this phenomenon as it reflects its ontogeny, organization and mature appearance (Arekal and Nagendran, 1975a,b). Because there is no double fertilization, nor endosperms development in Podostemaceae (Battaglia, 1980), it is assumed that nucellar plasmodium nourishes the development embryo (Davis, 1966; Arekal and Nagendran, 1975a) and protects it from desiccation (Magnus, 1913).

Although in subfamily 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 occur in Asian species such as *Griffithella hookeriana* (Razi, 1949), *Farmeria indica* and *Hydrobryopsis sessilis* (Arekal and Nagendran, 1975a), *Hydrobryum griffithii* (Nagendran et al., 1976), *Willisia selaginoides* (Arekal and Nagendran, 1976), *Polypleurum filifolium*, *P. dichotomum*, and *P. munnarense* (Nagendran et al., 1977) and *Zeylanidium olivaceum* and *Z. johnsonii* (Arekal and Nagendran 1977a). In contrast, it begins to develop at the time the megaspore mother cell undergoes meiosis-I in *Podostemum subulatus* (Nagendran et al., 1980), at early meiosis-I in *Dicreaea stylosa* (Mukkada, 1962a), after meiosis I in *Vanroyenella plumosa* (Murguía- Sánchez et al, 2002), at the stage when the embryo sac is mature in the Asian *Lawia zeylanica* (= *Dalzellia*) (Razi, 1949). In present study, the nucellar plasmodium begins to develop at early meiosis-I in *P. stylosum* var. *laciniata*, *Zeylanidium lichenoides* and *Willisia selaginoides*, it starts to developed at the two nucleate embryo sac stage. The narrowing of the embryo sac may be due to the inward growth of the inner integument.

In subfamily Tristichoideae, nucellar plasmodium formation also occurs at varying times, although in all cases it is after fertilization. In *Indotristicha ramosissima* (Chopra and Mukkada, 1966) it starts at the time of fertilization or early zygote development. Nucellar plasmodium formation begins immediately after fertilization in *Dalzellia zeylanica* (Arekal and Nagendran, 1977b) and in the pantropical species *Tristica trifaria* (Jäger-Zürn, 1967). So there is a wide range of variation in the initiation stage of nucellar plasmodium development inside Podostemacean family.

Embryologists have unreservedly pointed out the significance of the nucellar plasmodium for the growth of the gametophyte. This is valid because the inner integumentary cells in closer proximity to the developing proembryo stain negative for starch in contrast to the cells of the outer integumentary. This has been reported in Podostemaceae (Razi, 1955; Mukkada, 1969), diverse taxa of *Rhododendron* (Palser et al., 1992) and other angiospermous taxa (Palser et al., 1992; Jensen, 1965). There is provision to receive the growing embryo, which subsequently occupies the entire space of the nucellar plasmodium. Because of the limited size of the female gametophyte to provide enough space to the developing embryo, its area with that of nucellar plasmodium suffices.

The liquid medium of the nucellar plasmodium becomes useful during the embryogenic stages because the plants are suddenly exposed as water level in the streams and rivers subsides. This is an adaptation which enables a successful mode of life in the members of Podostemaceae in aquatic ecosystem. In this connection Arber (1920) calls the nucellar plasmodium (pseudo-embryo sac) as an ideal water reservoir. It follows that in the absence of the endosperm, an alternative, the nucellar plasmodium serve to conserve food materials, drawn by the developing embryo, suspended in its fluid of the cytoplasmic mass. This unit, also maintain the internal maternal ovular environment from collapsing through the tension effect of its fluid mass to counteract the inward pressure from the surrounding sporophytic tissues and other external sources where the plant grows. Probably, it furnishes certain morphogenetic substances necessary for differentiation of the developing embryo (Mukkada, 1969). These may include enzymes, growth hormones and osmoregulatory fluids.