4.3 EMBRYOLOGICAL STUDIES (Table 10)

4.3.1 Anther wall development and male gametophyte

During anther development, the hypodermal archesporial cells divide into the outer primary parietal cells and inner primary sporogenous cells (Figures 33A, 35A, 41A, 44A). The former divides periclinally and anticlinally to form the outer and inner secondary parietal cells (Figures 33B, 37A, 39B, 41B, 44B). The epidermal cells also divide anticlinally to increase the surface area for anther enlargement (Figure 37A).

The outer secondary parietal cells directly develop into an endothecium while the inner secondary parietal cells further divide into a single middle layer and tapetum. Thus, the anther wall development conforms to the Monocotyledonous type (Davis, 1960) (Figures 33C, 35B, 37B, 41A). Occasionally, the endothecium and middle layer are derived from the outer secondary parietal cells while the tapetum develops directly from the inner secondary parietal. This indicates that although the anther wall development usually conforms to the Monocotyledonous type, the Dicotyledonous type may occur (Figures 35C, 37C & 39C).

The mature anther wall consists of one layer of epidermis, endothecium, middle layer and multinucleate tapetum. The functional tapetum is glandular. The tapetal cells initially are uninucleate; they then become multinucleate (two to four nuclei) just before the microsporocytes undergo meiosis. The two to four nuclei either remain as separate nuclei or fuse into a large nucleus within the tapetal cells (Figures 37C & 38A).

The epidermis comprises a single layer of cells which is retained throughout anther development. Accumulation of tannin is usually observed in the epidermis of all the species studied (Figures 38F & 45B) but it is rare in *A. sessilis* and *A. brasiliana* (Figure 43A). The middle layer starts to degenerate at the pre-meiotic stage of the microsporocytes and completely degenerates when microspore tetrads are formed (Figures 38A–F).

The tapetum degenerates at different stages in the anther of *Alternanthera*. The tapetal cells are healthy with conspicuous nucleus at the pre-meiosis stage of microsporocytes. When the microspore tetrads are formed, the tapetal cells detach from each other and start showing degeneration. At this stage, the inner wall of the tapetal cells in all the species is devoid of ubisch granules deposition except in *A. bettzickiana* (Figure 45C). As the microspores develop into the signet-ring stage, the tapetal cells of *A. sessilis* are deeply stained until the nuclei are not seen and the inner walls of the tapetal cells are now deposited with ubisch granules (Figures 34E, 36A, 38I). However, the ubisch granules do not occur in the inner wall of the tapetal cells in *A. brasiliana* (Figures 42C, 43A & B). The tapetal cells of *A. paronychioides* and *A. ficoidea* are not deeply stained and occasionally their nucleoli are still visible (Figures 38I, 40C – D).

Eventually, the tapetum of *A. sessilis*, *A. paronychioides* and *A. ficoidea* completely degenerate when the anther is at the two-celled microspore stage (Figures 38J & 40E). On the other hand, the tapetum of *A. brasiliana* degenerate simultaneously with the degeneration of microspores at the post meiotic stage or during microsporogenesis (Figures 43A−C).

Soon after the formation of one-celled microspores, endothecial cells enlarge and become radially elongated. Fibrous thickenings are formed on the anticlinal and inner tangential walls in all the species studied except in the endothecium of *A. brasiliana*. At this stage, a large vacuole appears in the cells and the cytoplasm becomes sparse. When the two-celled microspores are developed, the remaining tapetum completely breaks down leaving only the epidermis and fibrous endothecium, and the inner wall of the endothecium is now covered with ubisch granules (Figures 38J & 40E).

Simultaneous with the development taking place in the anther wall, the sporogenous cells divide actively (Figure 44B); giving rise to the secondary sporogenous cells and they eventually differentiate into the microsporocytes. During

initiation of meiosis, callose begins to deposit around the microsporocytes and meiosis occurs (Figures 38C & D, 45A–B). Simultaneous cytokinesis gives rise to mostly tetrahedral and rarely isobilateral or decussate microspore tetrads (Figures 34D, 35D, 40B, 42B & 45C).

Later, the callose dissolves and releases the individual microspores into the locule. The microspores exhibit a dense cytoplasm with a prominent and centrally placed nucleus with a thin exine (Figures 34C, 38G & 42C). As the uninucleate microspores enlarge, the cells become vacuolated due to the utilization of cytoplasm in wall thickening. The nucleus is gradually pushed to the periphery, giving the microspores the characteristic signet-ring shape (Figures 34D, 36A, 38I & 40C).

The uninucleate microspores then undergo two mitotic divisions to give rise to mature pollen grains. The first division is defined as differentiation mitosis which results in two unequal cells, a small generative cell and a large vegetative cell while the exine and intine of the microspore wall become noticeable (Figures 34F, 36B, 40E). In the second mitotic division, the generative cell further divides into two male gametes. Initially, the male gametes are round in shape but they become spindle-shaped when the microspores are fully engorged with food reserves. Thus, the mature pollen grains are shed at the three-celled stage (Figure 38K).

Although microsporogenesis is relatively synchronous in the two microsporangia of an anther, asynchronous development is occasionally observed. For instance, meiosis (simultaneous cytokinesis) and post meiotic stage (microspores released from the tetrads) are observed in two different locules of the same anther of *A. paronychioides* (Figure 38L).

Alternanthera brasiliana does not produce any mature pollen grains as the anther is empty during anthesis (result obtained from Chapter 4.2.2). Abnormal

behaviour during microsporogenesis occurs at the first meiotic stage when most of their nuclei degenerate. At this stage, the tapetum completely degenerates (Figure 43C).

Rapid degeneration of the microspores has also been observed at post meiotic stages. After the microspores separate, the size of the microspore becomes irregular and the exine layer of the microspores is thin, does not differentiate and eventually the microspores degenerate. Occasionally, tapetum starts to show degeneration at the tetrahedral microspore stage (Figure 42B). The degenerated tapetum at this stage is devoid of ubisch granules on its inner wall (Figures 43A, B & D).

Similar to *A. brasiliana*, *A. bettzickiana* also does not produce mature pollen grains and the microspores are observed to behave abnormally after the post meiotic stage (Figure 46C). Failure in cytokinesis after meiosis II results in coenocytic microspores. After callose dissolution, binucleated or quadrinucleated coenocytic microspores in a wide range of shapes and sizes are produced (Figures 46A & B). Occasionally, more than one nucleolus is seen. Upon flower anthesis, these coenocytic microspores fail to develop into pollen grains.

Another type of abnormal microspores is produced other than coenocytic microspores. After callose dissolution, the individual microspores are surrounded by abundant ubisch granules. However, these microspores do not seem to develop an exine layer probably because they are surrounded by ubisch granules. Eventually, these microspores are devoid of cytoplasm, become vacuolated and degenerate (Figure 46C & D). Although the tapetum behaves normally and ubisch granules are present, the microspores not only are unable to develop into mature pollen grains, the exine is not formed as well.

4.3.2 Ovule development

The mature ovary is unicarpellary and unilocular with a basal ovule. The ovary consists of a single crassinucellate, bitegmic and campylotropous ovule with a long funiculus. Ovule development begins with initiation of the inner integument on both sides of an upright ovular primordium (Figure 90A). The upright ovule begins to curve when the outer integument on the side opposite to the funiculus is initiated (Figures 88A,, 89A & 91A). The curvature continues and at the same time, the outer integument near to the funiculus is initiated (Figures 88B & 90B). Subsequently, an upside-down ovule with a megasporocyte is formed (Figures 88C, 89B, 90C & 91D). In fact, the curvature does not stop at this stage but continue until a campylotropous ovule is formed (Figures 88D, 89D, 90D & 91B). By now, a mature embryo sac has developed. However, the female gametophyte of *A. bettzickiana* remains at the megasporocyte stage even after the campylotropous ovule is formed (Figure 91D). The style is short, hollow and capped by a papillate stigma.

Nucellus in the campylotropous ovule is curved and crassinucellate. Initially, the primary parietal cell is cut off (Figures 47A & B, 53B, 56A & 57A and become two layers when the female gametophyte is at the megasporocyte stage (Figure 54B). Further periclinal and anticlinal divisions give rise to three or four layers of massive nucellus at the micropylar region. The lateral side of the embryo sac is also surrounded by three or four layers of nucellus (Figures 48A & C). By now, a tetrad linear megaspore has developed.

When the embryo sac is enlarging and undergoing megagametogenesis, the nucellar cells in the inner layer of the lateral region adjoining the embryo sac start to degenerate (Figures 48D, 51B & 54).

Rapid degeneration of the nucellus at the chalazal region becomes noticeable while the embryo sac elongates into a horse-shoe shape (Figure 62E). By the time a

globular proembryo is developed, most of the nucellar cells at the chalazal region are degenerated. Nucellar cells at the lateral region also gradually degenerated and are completely consumed by the time the cotyledons are initiated (Figure 65C).

Nucellar cells at the micropylar region can persist much longer than those at the chalazal and lateral region. When the mature seed is formed, most of the nucellus cells at the micropylar region would have degenerated except the perisperm which is enclosed by the mature embryo. The perisperm cell is filled with starch grains that serve as food reserves for the mature embryo (Figures 71C, 75B, 76D & 78A).

Meanwhile, the nucellar cells in between the micropyle and the egg apparatus are morphologically different from those that surround the embryo sac. Two rows of conspicuous elongated nucellar cells are formed and serve as a pollen guide ensuring pollen tube transfer (Figures 49A, 55A & 59D). During fertilization, pollen tubes pass through these elongated nucellar cells and penetrate the female gametophyte via the micropyle (Figures 59A–C; 62B). After fertilization, these cells consisting of dense cytoplasm and nucleus elongate further. As a consequence, a strand with two rows of nucellar cells is noticeable, especially in *A. paronychioides* (Figures 64B & 76B).

Of the two integuments, the inner integument develops prior to the outer integument. The inner integument is initiated simultaneously on both sides of the ovular primordium, arising from the 2 to 3-celled dermal primordia (Figures 47B, 53B & 57A). Subsequently, the inner integument cells continue to divide anticlinally and periclinally until they completely cover the embryo sac at the megasporocyte stage. At this stage, the inner integument is 12 to 21-celled long (*A. sessilis* ‘Red’: 20 cells; *A. sessilis* ‘Green’: 21 cells; *A. paronychioides*: 15 cells; *A. ficoidea*: 12 cells) and 2-celled thick. Two to three larger and more massive cells are noticeable at the tip of the inner integument (Figure 54A).

When the upright ovule begins to curve, the outer integument on the side opposite to the funiculus is initiated (Figures 47A & 50B). Unlike the inner integument, the initiation of the outer integument does not happen simultaneously on both sides of the ovular primordium. In fact, the outer integument on the side opposite to the funiculus initiates prior to the side near to the funiculus. Similar to the inner integument, both layers of the outer integument arise from the 2 to 3-celled dermal primordia. The length of the outer integument on the side opposite to the funiculus is slightly shorter than the inner integument. By the time a megasporocyte is formed, the outer integument on the side opposite to the funiculus is 9 to19-celled long (*A. sessilis* 'Red': 19 cells; *A. sessilis* 'Green': 19 cells; *A. paronychioides*: 10 cells; *A. ficoidea*: 9 cells) and 2-celled thick. Before the ovule becomes upside down, the outer integument near to the funiculus is initiated (Figures 56C & 88B). This outer integument is shorter, only 8 to 9-celled long and 2-celled thick (Figures 48C & 50B).

As the ovule is of the campylotropous type, there is more growth on the side opposite to the funiculus resulting in greater curvature of the body of the ovule as well as the embryo sac. As a result, the ovule appears pressed against the funiculus. Therefore, only the inner integument alone contributes to the formation of micropyle as the outer integument is shorter than the inner integument. Occasionally, an air space is present in between the two integuments at the chalazal part of the young ovule (Figures 88D, 89C & 91D).

At the megasporocyte stage, the cells of both inner integument layers are rich in cytoplasmic material and possess conspicuous nuclei. Although the cells of both outer integuments layers are vacuolated, the conspicuous nuclei still persist (Figures 84A & 85A). As the ovule continues to curve after the megasporocyte stage, both integuments also divide periclinally and actively to increase the surface area for the ovule enlargement (Figures 84A–B, 86A). After dividing, the cells of the inner integument

enlarge tangentially whereas the cells of the outer integument enlarge radially (Figures 85C & 86B).

Floral nectaries are observed at the base of the stamens (Figure 92) which is evidenced by staining the flowers with 1% Sudan III solution (results obtained from Chapter 4). When a mature embryo sac has developed in the flower bud, the secretory parenchyma cells of the nectaries are filled with cytoplasm and have conspicuous nuclei. In addition, a few idioblasts with calcium oxalate crystals in the form of druses are found below the secretory parenchyma cells (Figure 92B). In the section of flowers, the secretory parenchyma cells have exuded nectar when a zygote is formed. By now, the cytoplasm of the secretory parenchyma cells has become less dense and a vacuole is formed. Most of the cells are also devoid of nuclei (Figure 92D).

4.3.3 Megasporogenesis and female gametophyte

A primary archesporial cell in the ovule begins to appear simultaneously with the differentiation of the inner integument initials (Figures 50A, 53A & 57C). The archesporial cell divides to form a primary parietal cell and a primary sporogenous cell (Figures 47A, 53B, 56A–C). A singular incident of more than one sporogenous cell is observed in *A. sessilis* ‘Red’ (Figure 47B).

The sporogenous cell gradually differentiates into the megasporocyte as its cytoplasm becomes denser and the nucleus enlarges. At this stage, the anther is at the late prophase stage (Figure 53C). The megasporocyte elongates and has a large nucleus with prominent nucleolus (Figures 47C, 50C, 54A & B, 56B & 57). The first meiotic division produces a dyad which later divides giving rise to a linear tetrad of four megaspores (Figure 48A). As a rule, the three megaspores nearest to the micropyle degenerate, leaving only the chalazal megaspore to undergo further development (Figures 48B & 51A). The nucleus of the functional megaspore is pushed in the

direction of the long axis of the cell and a vacuole appears below the nucleus (Figure 48B). The distance between the functional megaspore and the other three megaspores is distinct. At this stage, the anther is at the one- or two-celled microspore stage (Figure 48C).

After the first mitotic division in the functional megaspore, the two nuclei move apart to the opposite poles and the center of the embryo sac is occupied by a large vacuole. Most of the cytoplasm is aggregated around the nuclei but some may form a thin peripheral layer (Figures 48D, 51B & 54E). Both the nuclei divide again giving rise to a four-nucleate (Figures 51C & 54D) and finally an eight-nucleate embryo sac (Figure 52A). Occasionally, mitotic division in the two-nucleate embryo sac might not be synchronized and this results in a three-nucleate embryo sac (Figure 48E). Thus, the type of embryo sac development is of the monosporic *Polygonum* type (Maheshwari, 1950). At this stage, the anther has produced mature pollen grains at the three-celled stage.

Three nuclei from the chalazal region are the first to organize into antipodals. One nucleus from each pole migrates toward the middle of the embryo sac to become the two polar nuclei while the other three nuclei eventually differentiate into the egg apparatus at the micropylar region (Figures 49A & 52B).

The synergids are hooked and the filiform apparatus differentiates in the basal portion of the synergids (Figures 49D & 55A). A nucleus is located at the center of the cell with a large vacuole below it (Figures 49B & 52D). The pear-shaped egg cell is characterized by a nucleus at the chalazal end of the cell with a vacuole above it (Figure 52E); the nucleus of the egg cell is densely covered with cytoplasm (Figure 49C). The egg cell, which is smaller than the synergids initially, becomes longer than the synergids soon after differentiation (Figure 49D). In most cases, the egg cell is located in between

two synergids. A rare occurrence of an egg cell is observed on either side of the synergids as seen in *A. sessilis* 'Red' (Figure 49C).

Two polar nuclei in the center of the embryo sac fuse before fertilization. After fusion, the conspicuous secondary nucleus is located just below the egg cell (Figures 52E & 55B). An instance of three polar nuclei is observed in *A. sessilis* 'Green'. The extra nucleus is believed to have derived from the antipodals since there are only two antipodal cells at the chalazal region (Figure 52C).

In the present study, a few interesting observations have been noted. Probably owing to a lack of samples, meiotic and mitotic divisions during megasporogenesis and megagametogenesis have not been observed in *A. brasiliana*, *A. ficoidea* and *A. bettzickiana*. As a consequence, mature embryo sac is rarely observed in *A. brasiliana* and *A. ficoidea* while it is totally absent in *A. bettzickiana*.

Abnormal embryo sac is seen in these three species as well. The egg cell of *A. brasiliana* is not seen while the antipodals degenerate before the two polar nuclei are fused (Figure 58). Even if the egg cell is present, it eventually degenerate (Figure 80). This is different from *A. sessilis* and *A. paronychioides* where the antipodals only start to show degeneration after double fertilization. The mature embryo sac of *A. ficoidea* tends to degenerate before fertilization. For instance, the egg apparatus as well as the antipodals degenerate after the formation of the secondary nucleus (Figure 56C). In *A. bettzickiana*, the megasporocytes are poorly stained indicating degeneration.

4.3.4 Double fertilization

Although actual syngamy and triple fusion have not been observed, the presence of the primary endosperm and zygote suggest that this would have happened. In *A. sessilis* (both leaf forms), *A. paronychioides* and *A. ficoidea*, fertilization is deemed to happen within 24 hours after flower anthesis as zygote development has been observed.

As a rule, only one zygote is formed after fertilization. However, a single instance of three zygotes is recorded in *A. sessilis* 'Red'. The zygotes are at the resting stage while the primary endosperm nucleus divides, giving rise to six endosperm nuclei (Figure 61). Although the zygote is observed in *A. ficoidea*, it degenerates before developing into a proembryo (Figures 63A–B) but the primary endosperm nucleus remains healthy and does not degenerate.

Before fertilization, more than one pollen grains is observed on the stigma and the accessory pollen tubes make their way to the ovule through the hollow style. Eventually, the accessory pollen tubes pass through the nucellus and penetrate the megagametophyte via the micropyle (Figures 59 & 62A). Thus, fertilization is porogamous. While passing through the nucellus, the accessory pollen tube does not destroy the nucellar cells but make its way between them (Figures 59A & 62).

Normally, a single accessory pollen tube grows into one of the synergids and destroys it (Figure 59A). The pollen tube continues growing and reaches the basal part of the egg cell. The other synergid degenerates after the formation of the zygote and the remnants of the synergids and pollen tube persist until the four-celled linear proembryo stage. However, two exceptions are observed in *A. sessilis* 'Red'. In one single instance, the pollen tube does not grow into the synergid but in between the egg and one of the synergid (Figure 59B). Another instance shows two pollen tubes penetrating into the embryo sac but only one is successful in reaching the egg cell (Figure 59C).

The actual time of embryo sac elongation remain unknown. The embryo sac could start to elongate at these three possible stages: 1) before fertilization. 2) during fertilization. 3) soon after fertilization. The embryo sac elongates considerably into a horse-shoe shape. The ephemeral antipodals start to degenerate and are left in a lateral position due to the elongation of the embryo sac (Figures 60; 62D–E).

4.3.5 Development of fruit and seed

4.3.5.1 Endosperm and embryo

The endosperm development is of the *ab initi*o Nuclear type (Davis, 1966). The primary endosperm nucleus divides mitotically while the zygote undergoes a resting period. The divisions are slightly not synchronous since endosperm nuclei were observed in the metaphase as well as anaphase stage in a single embryo sac (Figure 161C). The endosperm nucleus is round with a conspicuous nucleolus. Two to three nucleoli are occasionally observed as well (Figure 65E). As the number of endosperm nuclei increases, the nuclei tend to concentrate around the proembryo and are unevenly distributed at the periphery of the elongated embryo sac while the centre of the embryo sac is occupied by a large vacuole (Figure 64A). When the seven-celled proembryo is formed, the accumulation of nuclei at the chalazal region is observed (Figure 64B). Frequently, the endosperm nuclei at the chalazal region are larger than those in the micropylar region (Figures 65A & E).

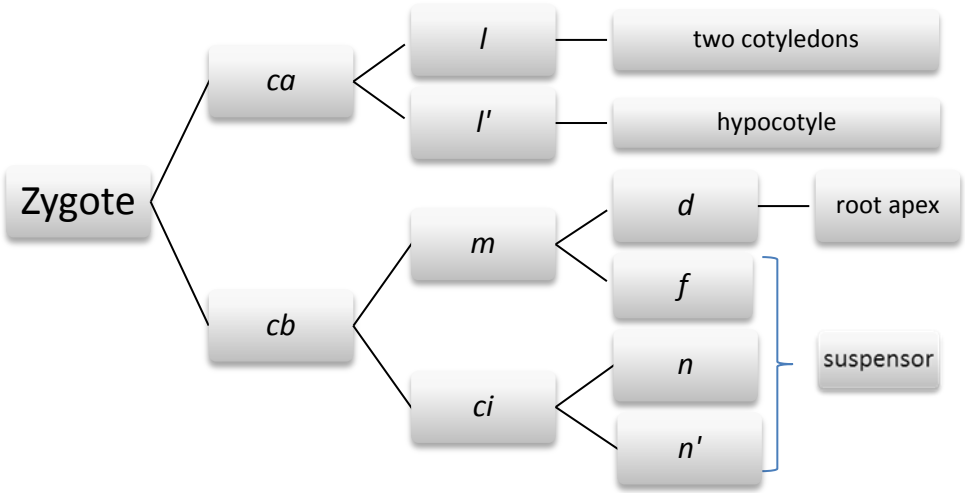
Wall formation in the nuclear endosperm begins at the micropylar region of the embryo sac when the late globular embryo is formed (Figures 65C & 66B). However, cell formation does not proceed to the chalazal region where the endosperm remains in the free nuclear state (Figure 65C). During the maturation of the embryo, almost all the endosperm is consumed, leaving only a single layer of cellular endosperm.

The zygote divides transversely giving rise to an apical cell (*ca*) and a basal cell (*cb*) (Figures 68C & 72A). The basal cell is slightly bigger than the apical cell. Another transverse division in the cells *ca* and *cb* results in a linear four-celled proembryo, designated as *l, l', m* and *ci* (Figure 68B). The division in cells *ca* and *cb* does not take place simultaneously, with the cell *cb* dividing before cell *ca* (Figures 68C & 72).

Transverse division in cell *m* results in *d* & *f* while the transverse division in cell *ci* results in *n* and *n'*. This is followed by another transverse division in either *n* or *n'*

giving rise to a linear proembryo of seven cells (*l, l', d, f, h, k, n'* or *l, l', d, f, n, o, p*) (Figures 69A& 73A).

Subsequent vertical divisions in *l* and *l'* result in a quadrant (Figures 69B & 73C). The quadrant embryo continues to divide vertically or obliquely giving rise to an octant embryo (Figures 69 & 73D). Thus, cell *l* is the initial to two cotyledons whereas the cell *l'* produces the hypocotyl. Meanwhile, the derivatives of cell *d* form the root apex and the remaining cells, *f, n* and *n',* form a six- to seven-celled long suspensor. Normally, the suspensor is uniseriate except at the basal end where it is multiseriate (Figure 70B). Since the embryo proper arises from both the cells *ca* and *cb,* embryogenesis of this genus is the transitional form between the Chenopodiad-type and Solanad-type (Johansen, 1950). This is shown below in a schematic manner.



As the proembryo develops into the young globular stage, the dermatogen and hypophysis are initiated. Periclinal divisions lead to the formation of outer and inner cells *l* and *l'*. The outer cells constitute the initial cells of the dermatogen and are distinguishable in the left and right part of the developing embryo (Figures 70A, 70A & 76B). These initial cells of the dermatogen continue to divide anticlinally and gradually

differentiate into the mature dermatogen (Figures 70D & 74). Meanwhile, the hypophysis consists of two cells and is located at the border between the suspensor and the proembryo. The hypophysis is different from the dermatogen in having lens-shaped cells and is detached from the upper cell of the suspensor (Figure 70C).

Histogenic embryo differentiation is not observed until the cotyledons are initiated in the heart-shaped embryo (Figures 71A & 76C). The cylindrical shaped procambial cells are discernible and are located in the hypocotyl of the embryo axis (Figures 71B & 75A). As the embryo develops, the procambial cells extend from the central column of the embryo axis into the cotyledons (Figures 78B & C). The procambial cells are rich in proteins. Eventually, a mature embryo is characterized as dicotyledonous, curved, annular and encloses the perisperm. The cotyledons are longer than the hypocotyl (Figures 71C, 75B, 76D & 78).

A single case of abnormal embryo development has been observed in *A. paronychioides.* A young globular embryo was seen in the elongated embryo sac although the primary endosperm nucleus had not yet developed into the endosperm. Furthermore, the degenerating antipodals and the remnants of synergids were also observed (Figure 77).

Even though viable pollen grains and embryo sac are not produced, mature embryo is observed in *A. brasiliana*. The embryo is developed from the nucellar embryo initial cells surrounding the embryo sac. These cells are morphologically distinguished from the outer nucellar cells in having conspicuous nuclei, dense cytoplasm and starch (Figure 79). Therefore, *A. brasiliana* is an apomict that exhibits adventive embryony (sporophytic apomixis).

The nucellar embryo initial cells are seen when the egg apparatus shows degeneration (Figure 80). The embryo initial cells divide vertically and horizontally, producing buds which grow into the embryo sac (Figure 81A). The divisions in the buds

are irregular and buds of different shapes are produced. Nucellar embryo buds are formed in different areas of the embryo sac such as the micropylar or chalazal region as well as along the length of the embryo sac (Figure 81B).

As the development continues, one of these nucellar embryo buds attains maturity and grows into a mature embryo. However, there is a possibility that more than one nucellar embryo could develop. This is evidenced by two zygotes, two globular proembryos and two embryos in the embryo sac (Figures 81B, 82B & 83A). When polyembryony occurs within a mature embryo sac, the two nucellar embryos differ in size. Often, one of the embryos is very much longer than the other one (Figure 83). This observation is in accordance with the results of seed germination as reported in Chapter 4.7.

Nucellar embryo development of *A. brasiliana* is morphologically similar to the sexually derived embryo development as seen in *A. sessilis* and *A. paronychioides*. The mature nucellar embryo is characterized as dicotyledonous, curved, annular and encloses the perisperm. The cotyledons are longer than the hypocotyl. However, the nucellar embryo of *A. brasiliana* does not have suspensors throughout the development of the embryos. Furthermore, the nucellar globular proembryo of *A. brasiliana* is situated further away from the micropyle when compared to *A. sessilis* (*A. sessilis*: Figures 64C & 65C; *A. brasiliana*: Figures 82A & C).

Although double fertilization does not occur in *A. brasiliana*, nuclear endosperm is able to develop from the two polar nuclei. Before the degeneration of the egg apparatus takes place, the two polar nuclei are closely arranged (Figure 58). When the egg apparatus starts to degenerate, nuclear endosperm is formed in order to nourish the adventive embryo (Figure 80B). Thus, the endosperm development conforms to the autonomous type. Similar to the other species studied, the wall formation commences at

the globular stage from the micropylar region but the nuclear endosperm remains free at the chalazal region (Figure 67). During the maturation of the embryo, almost all the endosperm is consumed, leaving only a single layer of cellular endosperm.

4.3.5.2 Seed coat

Generally, the seed coat development coincides with the formation of the zygote. Out of the two integuments, the inner integument degenerates before the outer integument. At the stage of the four-celled proembryo, the cells of both inner integument layers shrink; become vacuolated and are devoid of nuclei (Figures 84D & 85D). When the proembryo is at the young globular stage, the inner layer of the inner integument completely degenerates except the cells that make up the micropyle (Figures 84F & 85F). These cells are eventually deposited with granular contents (Figures 86D & E). Later at the torpedo proembryo stage, the outer layer of the inner integument will have degenerated (Figures 84G, 85G & 87C).

The inner integument of *A. paronychioides* behaves differently from the other species in two ways. Firstly, the outer layer is degenerated when the heart-shaped proembryo is formed whereas in the other species, the outer layer is degenerated at the four-celled proembryo stage. Secondly, a layer of cuticle in between the inner layer of the inner integument and nucellus is observed only in *A. paronychioides* (Figures 86D & E).

While the degeneration of the inner integument is observed at the octant stage, the outer layer of the outer integument undergoes obvious cytological change. Initially, the cells of the outer layer of the outer integument are characterized by the random deposition of small granular contents (Figures 84E, 85E, 86D & 87B). As the proembryo develops, the cells of the outer layer of the outer integument become more

vacuolated and elongated. At the same time, granular contents accumulate at the periphery, the center or across the whole cell (Figures 84F–H, 85F & H, 86E & 87).

The outer periclinal walls of the outer layer of the outer integument only become thickened at the torpedo proembryo stage (Figures 84G, 85G & 87C). When the mature seed is fully developed, thickenings of the periclinal walls become discernible especially when tanniferous materials grow further downwards. In fact, *A. sessilis*, both the red and green leaf forms, has the most distinct cell wall lignification (84G, 85H & 87D).

Simultaneous with the changes taking place in the outer layer of the outer integument, the inner layer of the same integument becomes elongated, vacuolated, devoid of nuclei and is degenerated when a mature fruit is formed. Therefore, only the outer layer of the outer integument and a small part of the inner layer of the inner integument that make up the micropyle contribute to the formation of the seed coat.