# EMBRYOLOGICAL STUDIES IN *AMARANTHUS LEUCOCARPUS* S. WATS

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**Abstract:** Embryological observations in *Amaranthus leucocarpus* indicate absence of a distinct endothecium. Small globules occur beneath the tapetal cells. The pollen are shed at the three-celled stage. The ovule is amphitropous, a clear space is present between the two integuments and nucleus. Embryo sac development is of the polygonum type. Antipodals persist till about the 4-celled stage of the endosperm. The radicular apex is established by the late globular stage of the embryo. Shoot apex differentiation commences by the torpedo stage of the embryo.

## INTRODUCTION

Quite a few members of the Amaranthaceae have been investigated for embryology. Woodcock (1931) studied the development of *Amaranthus caudatus*. Naithani (1933), Joshi and Rao (1934) investigated the embryology of *Digera arvensis*. Which was reinvestigated by Puri and Singh (1935). Joshi and Kajale (1937) Dambroise (1947) have given a detailed account of the endosperm development of many members of Centrospermales including *Amaranthus retroflexus* and *Celosia cristata*. Bakshi (1952) recorded embryological observations in *Psilostachys sericea*. Sachar and Murgai (1958) recorded embryological observations in *Aerua tomentosa*. Padhye (1962) reported the solanad type of embryo development in *Gomphrena celosioides*. Aruna (1968) studied the seed development in *Celosia cristata*. In all these works *Amaranthus leucocarpus* has not been figured for detailed embryological studies.

## MATERIALS AND METHODS

Materials for the present study were collected from the plants grown in the Botanical Garden of B. I. T. S. Pilani. Record of dates was kept during the fixation of material.

The material was fixed in formalin-acetic-alcohol and the usual procedures of dehydration and paraffin embedding were followed. Sections were cut at 6-8 microns and were stained with safranin-fast green combination.

## OBSERVATIONS AND RESULTS

**Microsporogenesis:** The young anther lobe shows a hypodermal archesporium, 6-7 cells across in a transverse section and about 9 cells in longitudinal section (Plate I-A, B). The archesporial cells enlarge and become richly protoplasmic. They divide periclinally to form an outer parietal layer and inner sporogenus layer. The periclinal division does not take place simultaneously in all cells of archesporium (Plate I-A). The parietal layer divides giving rise to the endothecium and an

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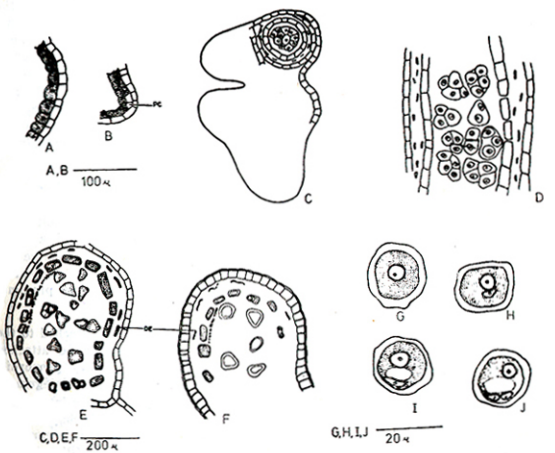


Plate I. *Microsporogenesis*

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| A, B | Young anther lobe showing hypodermal archesporium. |
| C | L. S. microsporangium showing microspore mother cells, remnants of middle layer and conspicuous tapetum. |
| D, E, F | L. S. portion of microsporangium showing the separation of tapetal cells and the degenerating endothecium. |
| G, H, I, J | Development of male gametophyte. |

inner layer which divides once more to produce a single middle layer and the tapetum. The middle layer degenerates as early as the microspore mother cell stage. Its disintegrated remains are seen above the tapetum (Plate I–C). The separation of tapetal cells from one another commences at a tetrad stage and continues until the microspores separate from one another (Plate I–D, E). At a stage when the microspore nuclei are ready for division to form generative cell, just beneath the tapetal cells appear granular matter which becomes denser by the time the male cells are formed. As the granular matter becomes denser the contents of tapetal cells become less dense. The granular material stains red by safranin. By this stage even the endothecium disintegrates (Plate I–E, F). Thus the endothecium in *A. leucocarpus* is ephimeral. In certain preparations this layer is observed to remain intact, but characteristic fibrous thickening are not formed. In both cases the epidermal cells of the microsporangium become larger, slightly radially elongated and cutinized (Plate I–F). The microspore tetrads are decussate and tetrahedral (Plate I–D). Reduction divisions are simultaneous and cytokinesis takes place by furrowing.

**Male gametophyte:** The uninucleate pollen grain is full of cytoplasm. No vacuole is formed, hence the microspore nucleus divides in the central region of the microspore to produce a large vegetative cell and a small generative cell (Plate I–G, H). Between the vegetative nucleus and small generative nuclei a small vacuole is formed which further increases in size and pushes the generative cell toward the periphery (Plate I–I). The lenticular generative cell divides to give rise to two male cells which are pointed at their distal ends and flat on their proximal sides (Plate I–I, J).

**The ovule:** There is a single, bitegmic crassi-nucellate ovule attached to the base of the ovary. In certain preparations ovules have been observed attached laterally. Initially the ovule is orthotropus with visible integumentary primordia, but it curves to attain an anatropus condition. At this stage the inner integument surrounds the nucellus and forms the micropyle (Plate II–A). The inner integument is composed of two or three cell layers and the tips are slightly club shaped. The outer integument develops slowly and does not reach the level of inner integument. It is also composed of two cell layers with slightly tapering ends (Plate II–A). During the embryo sac stage there is more growth on the side opposite to the funicle resulting in greater curvature of body of the ovule as well as the embryo sac (Plate II–B). As a result of the pressure due to more growth on the opposite side the body of the ovule appears pressed against the funiculus. This is evidenced by the compression of the cells at the chalazal region. Thus the ovule becomes amphitropous (Plate II–C). Both the integuments and nucellus are separated by a distinct air space running throughout the length of the ovule (Plate II–D).

**Megasporogenesis:** A hypodermal archesporium cell differentiates in the young nucellus when the ovule is at a stage between orthotropous and anatropous (Plate II–E). The archesporial cell divides to form an outer parietal and inner megaspore mother cell. The parietal cell divides anticlinally and periclinally to give rise to a massive parietal tissue, the cells of which are arranged in radiating rows (Plate II–D). The megaspore mother cell enlarges considerably and divides forming two diad cells which divide once again to form a linear row of four megaspores (Plate

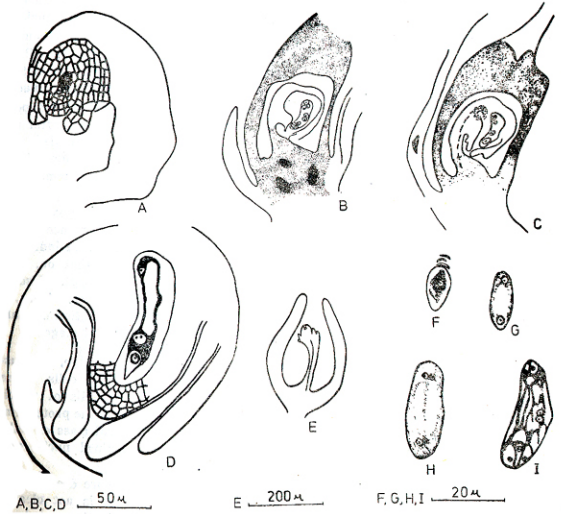


Plate II. *Megasporogenesis*

A, B, C, D Showing the change of the ovular position from orthotropous to anatropous condition.
E Orthotropous ovule with an archesporial cell.
F. G. H, I Development of female gametophyte.

II–A). The chalazal megaspore enlarges and gives rise to an embryo sac while the other degenerates.

**Female gametophyte:** The functional megaspore becomes vacuolated and its nucleus comes to lie in the centre. It soon divides to give rise to a two-nucleate embryo sac (Plate II–F, G). These nuclei are pushed to the poles by the formation of a central vacuole and each of them divides to give rise to a four nucleate embryo sac. Further divisions of the four nuclei give rise to an eight-nucleate embryo sac. These nuclei are pushed to the poles by the formation of a central vacuole and each of them divides to give rise to a four nucleate embryo sac (Plate II–G, H). Thus the embryo sac development is of the polygonum type. The mature embryo sac is curved and synergids are slightly hooked. The two polar nuclei fuse in the middle part of the embryo sac (Plate II–B, I). Synergids disappear by the time secondary nucleus comes to lie beneath the egg. It is only after the disappearance of synergids that the flask-shaped nature of the egg is revealed. At this stage the antipodals separate from one another and assume a triangular shape (Plate II–D). The antipodals have been observed to persist till the four-nucleate stage of the endosperm.

By fixing the material for embryology at periodic intervals it was possible to find out approximately how many days are involved from the appearance of the anther archesporium to pollination. The material fixed on 22nd Sept. '71 indicated archesporium, that fixed on 23rd Sept. '71 showed the tetrads and that on, 25th Sept. '71, indicated three celled pollen. In the material fixed on 25th Sept. '71 some pollen grains were observed on the stigma of the adjacent female flower. Thus, it takes approximately four days from the archesporial stage to the formation of male gametophyte, and pollination. The pollen was shed at the three celled stage.

**Embryo development and histogenesis:** In the early globular stage, the suspensor is short and the hypophysis is composed of two jutaposed cells. The terminal part of the developing embryo has a surface layer, the cells of which exhibit mainly anticlinal divisions. This layer, therefore, can be called the protoderm. At this stage tissue differentiation is not observed in the central mass of cells. The cells of this region divide in all planes and have deeper stained nuclei and cytoplasm than those of the protoderm layer (Plate III–A).

In the late globular stage, the protoderm cells are stained more deeply than the cells at the centre of the embryo. The embryonic shoot apex is not clearly distinguishable at this stage. At the proximal side, the cells within the protoderm differentiate into an outer embryonic cortex of three to four layers and a central embryonic stele. The central zone contains elongated highly stained cells which are the initials of the procambial cells. Unlike the embryonic shoot apex, the root apical region becomes more clearly delineated at this stage. The periblem and plerome initials are arranged in two superimposed tiers and are hexagonal in shape. The cells towards the flanks of these tiers exhibit the Korper type of division (Plate III–B).

During the heart-shaped stage of the embryo, the shoot apex appears as a small dome between the cotyledonary shoulders. The cells of the shoot apex are more densely stained than the central cells. The tunica of the shoot apex is prominent at this stage. The root organization also is more clearly visible at this stage (Plate III–C).

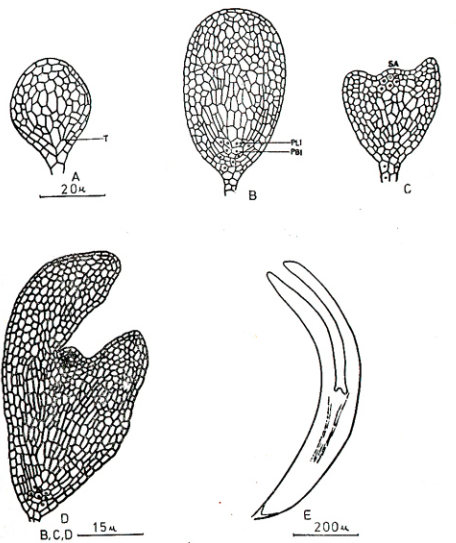


Plate III. Development of Embryo

A Early globular stage.
B Late globular stage.
C Heart shaped stage.
D Torpedo stage.
E Mature embryo.
T T-division. PLI Plerome initials. PBI Periblem initials. SA Shoot apex.
SAI Sub-apical initials of cotyledons.

In the torpedo-shaped stage of the embryo, the cotyledons have grown more in size by the activity of the sub-apical initials (Plate III–D). The radicular apex becomes more extensive at this stage. Kappe type of divisions are seen at the suspensor end. These divisions lead to two or three layers of cells which constitute the periphery of the root cap. Korper type of divisions in the formation of periblem and plerome initials results in the formation of periblem and plerome of the root.

In the mature embryo (Plate III–E) the outermost layer of the shoot apex is differentiated into a single layered tunica. The cells of this layer divide only in anticlinal planes and constitute the corpus. At the suspensor end the embryonic root apex also attains clear zonation.

## DISCUSSION

The presence of globular bodies just beneath the tapetal cells has been recorded in *Digera arvensis* (Puri and Sigh, 1935; Kajale, 1940) and in *Psilostachys sericea* (Bakshi, 1952). Such deeply stained globular, rather granular matter appears in *Amaranthus leucocarpus* also. The exact nature and function of these globules are not properly understood, but it is believed that these globules have a role in the formation of the exine of the microspores. Well developed endothecium with fibrous thickenings has been reported for many of Amaranthaceae (Puri and Singh 1935); however in *Amaranthus leucocarpus* the endothecium is short lived, it degenerates by the microspore stage and till then the cells do not develop any fibrillar thickening. The epidermal cells are slightly elongated and become cutinized.

Generally, prior to the division of the microspore nucleus to give rise to a generative cell, the nucleus is pushed to the periphery by the formation of a vacuole (Maheshwari, 1950). In *Amaranthus leucocarpus*, however, the nucleus divides in the centre to form a generative and vegetative nuclei and then a small vacuole appears between the two. This vacuole enlarges pushing the generative nucleus to the periphery.

The ovule in *Amaranthus leucocarpus* is amphitropous by bitegmic and crassinucellate. Bakshi (1952) Padhye (1962) have reported circinotropous ovules in *Psilostachys sericea* and *Gomphrena celoceaoides* respectively. The ovule in Amaranthaceae is basal but in *Amaranthus leucocarpus* in addition to the usual basal ovule certain sections revealed a lateral (peripheral) position. Does this indicate that basal placentation is derived from parietal? There is a clear space between the two integuments and also between the inner integument and nucellus. Similar observations have been recorded for *Psilostachys sericea* (Bakshi 1952). However *Digera arvensis* does not show any such space (Joshi and Rao 1934; Puri and Singh, 1935). The behaviour of antipodal cells is variable in the members of Amaranthaceae. In *Amaranthus leucocarpus* the antipodal cells persist till about the four-nucleate stage of the endosperm. The persistance of antipodals has been recorded from the other members of Amaranthaceae (Joshi 1935; Kajale, 1935, 1937, 1940). In *Pupalia lappaceae* the antipodals divide to form a mass of 30–40 cells (Kajale, 1940). Bakshi (1952) reports ephimeral antipodals in *Psilostachys sericea.*

Only a few studies on the histogenesis of embryo have emphasized the anatomy of apical meristems. The studies of Schoff (1943), Allen (1947) and Spurr (1949) on gymnosperms and of Boke (1944) and Guttenberg (1961) on angiosperms are of the opinion that the root pole is the first part of the embryo to become differentiated.

In contrast Meyer (1958) and Mahlberg (1960) have put forward the view that the shoot pole is the first to become distinct in the embryo of the McIntosh apple and *Nerium* respectively. Similar observation has been recorded by Padmanabhan (1964, 1967) for *Avicenia* and *Epithema*, and Swamy and Padmanabhan (1964) for *Sphenclea*. In *Amaranthus leucocarpus* the radicle initials become distinct at the globular stage of the embryo. The developmental studies in *Amaranthus leucocarpus* clearly indicate that the appearence of the epicotyl apex is quite late in the ontogeny of the embryo. It is therefore, not possible to agree with Mahlberg (1960) and Padmanabhan (1967) who stated that the epicotyl apex is the first to be delimited in the embryo.

Brown (1960) has expressed his view that the cotyledons are the embryonic organs, their resemblence to a foliage leaf being superficial. Padmanabhan (1967) in *Epithema* and Kaplan (1969) in *Dowingia* have regarded the cotyledons as the first foliage leaves. The method of origin, development and vascularization of the cotyledons tend to support the view that the cotyledons are the first foliage leaves. The presence of the buds in the axil of cotyledons in *Amaranthus leucocarpus* also strongly suggests the foliar nature of the cotyledons.

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